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Reproduction and rearing of advanced northern pike (*Esox lucius* L.) fry

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I. The objective of the methodology

The objective of this certified methodology is to describe and explain, new procedures of controlled reproduction of broodstock pike (*Esox lucius L.*) optimizing synchronization of broodstock spawning, the procedure of artificial fertilization and incubation of eggs and obtaining good-quality and highly viable larvae of this species. Another objective of this specialized methodology is to describe modern and effective production methods of juvenile pike to the advanced fry of a total length (TL) of 30 – 50 mm which are subsequently used for rearing the older age categories of fish until they reach the category of commercial fish with a body weight ranging from 1 to 3 kg. It is supposed that this scientific publication could help Czech production fisheries to increase the efficiency and production of pike in the Czech Republic which may diversity the Czech fishery production.

II. Description of the methodology

2.1. Commercial importance of pike in Europe

Pike (Fig. 1) is a commercially attractive piscivorous fish species occurring in many different freshwater biotopes of the Northern hemisphere (Crossman, 1996). This species is also very popular among sport fishermen (Mann, 1996). The popularity is, first of all, caused by a wide-spread occurrence of pike due to its high adaptability to various natural biotopes (Crossman, 1996). Secondly, it is the fish predator that offers a highly interesting sport fishing experience (Lusk and Krčál, 1982; Mann, 1996). Thirdly, pike is popular owing to its high quality muscle mass that has a little fishy taste, is highly dietary and easy to digest (Lusk and Krčál, 1982). On the other hand, it must be mentioned, that in Europe pike represents a supplementary fish species which is, in no case, caught by sport fishermen on a mass scale. As a matter of fact, pike is a an apex predator in open waters which occurs in given localities in relatively low densities (3–4 $kg.ha^{-1}$) and its density is often even lower than 1 $kg.ha^{-1}$. However, this assertion is not generally valid in Finland, Russia, and Ukraine where the mentioned fish species is abundantly caught by sport fishermen (Mann, 1996). Pike abundance in the above-mentioned countries is given by a large number and the total area of water surface of different lakes or reservoirs (Crossman, 1996).

In addition to sport fishing, pike is frequently used in extensive polyculture fish farming in ponds. Under such conditions, pike adopts the role of a predator whose task is to suppress occurrence of small and less commercially important cyprinid fish species, such as roach (*Rutilus rutilus*), common bream (*Abramis brama*), silver bream (*Abramis bjoerkna),* rudd (*Scardinius erythrophthalmus*), topmouth gudgeon (*Pseudorasbora parva*) and gibel carp (*Carassius gibelio*). Suppression of occurrence of these less valuable fish species in ponds reduces their competition against main, commercially most significant, fish species – common carp (*Cyprinus carpio*) (Adámek et al., 2010). This use of pike in pond farming maintains a high and an effective production of carp in ponds and its second contribution lies in an assessment of biomass of less commercially important fish species in a form of a biomass growth of economically highly valuable species (Lusk and Krčál, 1982; Dubský, 1998).

A strong predation pressure exerted by pike is used in controlled stocks during so-called biomanipulations in water supply reservoirs. The principle of controlled fish stocks is to support populations of piscivorous fish species that are able to control biomass of small planktonophagous fish species. A decreased occurrence and biomass of these small planktonophagous species reduces their grazing pressure on filtering zooplankton. This fact

enables greater development of zooplankton which effectively reduces development of phytoplankton, or more precisely, massive occurrence of so-called water bloom. This measure can ensure a relatively good water quality in water supply reservoirs. The assertion, however, is valid only if a low final fish biomass (lower than 100 $kg.ha^{-1}$) is kept in reservoirs and the aquatic environment is burdened with a low content of phosphorus at a mesotrophic level (Adámek et al., 2010).

Fig. 1. *Broodstock pike (Esox lucius L.).*

2.2. Current methods of a commercial fish production and its amount in Europe and the Czech Republic

At present, commercial pike is produced in Europe mainly in two ways. The first method involves capturing wild fish in large lakes and rivers. In this way, an amount of 17 700–24 500 tonnes of pike was produced per year over the past decade in Europe. The biggest European producers of fish obtained in this way are, Russia (8 000–16 000 t) and Finland (6 500–8 300 t). Other significant producers of pike by means of capturing are the following European countries (data in brackets represent an average annual production of pike in the course of the past decade): Poland (250–325 t), Hungary (170–280 t), Germany (170–210 t), the Czech Republic (140–180 t), Estonia (95–200 t) and Serbia (70–220 t) (FAO, 2013a). In the Czech Republic, pike are primarily caught from open waters by sport fishermen. Annual catches of pike carried out in this way amounted to a level of 120–170 tonnes between 2008–2010 (Ženíšková and Gall, 2011).

In addition to capturing pike from open waters, this fish species has traditionally been produced and reared in the above-mentioned extensive pond farming. Owing to this type of fish farming, an amount of 220–850 tonnes of pike is annually produced for the fish market in Europe which represents only about 3–10% of the production obtained by catching. The most significant producers of pike by means of pond fish farming in the past decade were the following countries: Russia (4–280 t), Poland (0–170 t), the Czech Republic (60–110 t), Belarus (40–120 t) and Hungary (30–80 t) (FAO, 2013b).

The biggest Czech producer of commercial pike is the Třeboň Fishery plc. with the total production of 22 tonnes in 2011. Other significant Czech producers of pike are the following fisheries and the data in brackets provide the annual production in 2011: Kardašova Řečice Fishery Ltd. (8.1 t), Hluboká Fishery Ltd. (4.8 t), Mariánské Lázně Fishery Ltd. (4.6 t) and Chlumec nad Cidlinou Fishery plc. (4.2 t). Advanced pike fry (TL = 30–50 mm) is stocked into production ponds at very low densities around 100–400 pcs.ha^{-1} at the beginning of a 2–4 year production cycle (Hamáčková, 1987). An average final biomass of pike reared in polycultural pond stocks of commercial fish ranges from 0.7 to 16.0 kg.ha^{-1} under the Czech fishery conditions (Kratochvíl, 2012). These very low production densities are influenced by a high predation pressure of pike exerted against fish population in given ponds including a cannibalism among pike (Lusk and Krčál, 1982). On this account, ponds have a very limited production capacity for effective pike farming.

2.3. Factors considerably limiting the current production

The production of commercial pike within the scope of the Czech fishery has been considerably limited both by the biology of the species itself and by insufficient or suboptimal farming conditions (Lusk and Krčál, 1982; Policar, 2012a,b). Biological qualities of pike limiting its rearing comprise: a long spawning period of hormonally untreated broodstock (Policar, 2012a), production of a very low volume of sperm (Linhart, 1984; Billard, 1996; Hulák et al., 2008a), contamination of extracted sperm with urine causing its reduced usability for artificial fertilization of eggs (Berka and Hamáčková, 1980; Billard, 1996; Hulák et al., 2008a), application of testicular sperm causing a loss of broodstock males for further use (Billard, 1996; Lahnsteiner et al., 1998), variable egg quality (Lusk and Krčál, 1982; Policar, 2012a; Švinger et al., 2012), susceptibility of fertilized eggs and embryos to manipulation or unsuccessful incubation (Berka and Hamáčková, 1980), high level of cannibalism (Lusk and Krčál, 1982; Kucska et al., 2005; Szczepkowski, 2009) and high territoriality of fish occurring from juvenile stages (Berka and Hamáčková, 1980; Lusk and Krčál, 1982).

Rearing conditions or interventions limiting pike production are: used suboptimal water temperature for rearing (Szczepkowski, 2009; Policar, 2012b), insufficient amount of suitable food (Berka and Hamáčková, 1980; Hamáčková, 1987) and a low density of reared fish (Lusk and Krčál, 1982; Hamáčková, 1987).

2.4. General reproductive characteristics

In the climatic conditions of Czech Republic, pike reproduce the most often from the end of February until the end of March, or until the beginning of April (Kouřil and Hamáčková, 1975; Dubský, 1998; Bondarenko et al., 2012b). Reproduction takes place at water temperature of 7–10 °C and spawning period is finished when water temperature reaches 14 °C (Lusk and Krčál, 1982; Westers and Stickney, 1993).

Pike belongs to phytophilous species which lay its fertilized eggs onto the submerged macro vegetation, especially fine-leaved aquatic macrophytes. Natural spawning environment of pike is represented by shallow warm sections of ponds and rivers that can even be periodically flooded. Eggs are sticky, therefore, they are firmly stuck to a plant substrate. Natural reproduction of pike is endangered by a low water level or its frequent fluctuation (especially in valley reservoirs) and an insufficient amount of suitable spawning substrate (Lusk and Krčál, 1982).

Sexual maturation of broodstock is dependent on temperature conditions and a food offer available in localities (Billard, 1996). In Spain, where pike was stocked and subsequently

acclimatized, males as well as females mature already at the end of the first year of life (Crossman, 1996). It is also possible to encounter one-year-old sexually mature fish even in the Central European conditions (Kouřil and Hamáčková, 1975; Lusk and Krčál, 1982), in the Czech climatic conditions, pike maturate at the end of the second or third, and sometimes even fourth, year of life (Billard, 1996). Males mature earlier than females when they reach a total length of 180 mm as early as in the first year of life. Females can be sexually mature in the second year of their life when they achieve a TL of 260 mm (Billard, 1996; Hubenova and Zaikov, 2007).

Sexual organs (ovaries and testicles) of both genders are paired, placed in abdominal part along the external side of kidneys (Kouřil et al., 1976). Ovaries are typically pear-shaped and before the spawning period they turn orange which characterizes final stages of oocytes. Testicles are elongated, white or cream-coloured (Lenhard and Cakic, 2002). Male's testicles intensively grow and develop already at the end of summer. Female's oocytes in ovaries intensively develop during the winter period until the beginning of spring (from November till March, or the beginning of April) when the final oocyte stages occupy up to 95% of an ovarian volume. The GSI (the gonadosomatic index expresses a percentage of a gonad weight compare to body weight) in females ranges in summer around 1–2%, in autumn, it is 5% and in winter, it reach up to 10–12%. In spring, before spawning, the female's GSI amounted to 18–20%. In this period, ovaries fill in a considerable part of the abdominal cavity (Billard, 1983; 1996).

Sexual dimorphism, as in other fish species inhabiting the temperate zone, is not too noticeable during the spawning period (Lusk and Krčál, 1982; Dubský, 1998). Females are distinguished only by enlarged abdominal areas. Gender can be recognized according to a shape of genito-urinary papilla (Casselman, 1974; Billard, 1983) which has also been confirmed by our present experience in gender distinguishing in the spawning period. Male's genito-urinary papilla is of a line shape, it is narrow and indistinctive, while female's papilla is fan-shaped, well-perfused with blood and reddish (Fig. 2).

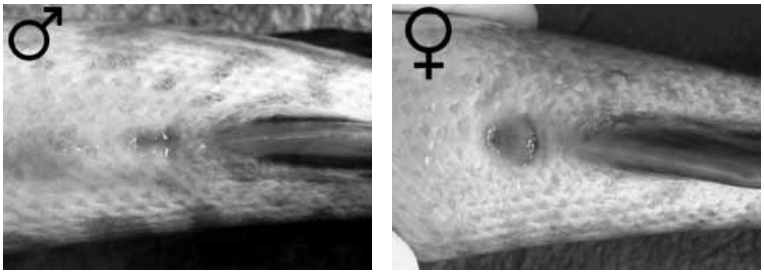

Fig. 2. *Distinguishing gender of pike (Esox lucius L.) by means of a shape and a state of a genito-urinary papilla, male (left) and female (right) (photo: V. Bondarenko).*

2.5. Marking and evidence of broodstock

In order to individually mark particular broodstock, either passive integrated transponders (PIT), sometimes called only "chips" or "microchips" (Rodina and Flajšhans, 2008), or metal fin clips are used (Fig. 3). Individual marking of fish is very important for fishery purposes in order to properly record spawning activities, fertility and survival of fish after the spawning period and, subsequently, also for evidenceof repeated spawnings in the following years. Microchips with a numerical code are the most often implanted in dorsal muscles on the left-hand side of a fish, approximately at a level of the first hard dorsal fin ray in a cranial direction 1 to 1.5 cm

deep under an angle of 30° by means of a sterile single-use or a reusable implanting device. The second mentioned method of fish marking is often employed in French fish hatcheries where certain broodstock are marked with a fin clips with a number of fish attached to dorsal or ventral fin.

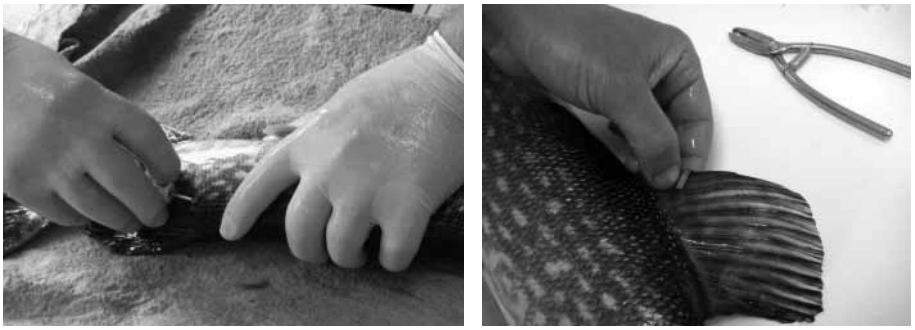

Fig. 3. *Marking of broodstock pike (Esox lucius L.) with a PIT microchip (left) and a fin clips with a number (right) (photo: J. Křišťan).*

It is important to calm fish down before the marking itself with the use of anaesthetics (Fig. 4). Clove oil at a dose of 0.04 $ml.l^{-1}$ is frequently used for pike as well as other fish species (Švinger et al., 2012). After marking, it is advisable to place a given fish into a preventive anti-fungal bath containing a potassium permanganate solution in a concentration of 0.1 $g.l^{-1}$ with an exposure time of 10–15 minutes (Fig. 5). This bath serves as prevention against secondary fungal infection before subsequent stocking of fish into troughs in a hatchery just before the fish spawning period itself (Policar et al., 2011a).

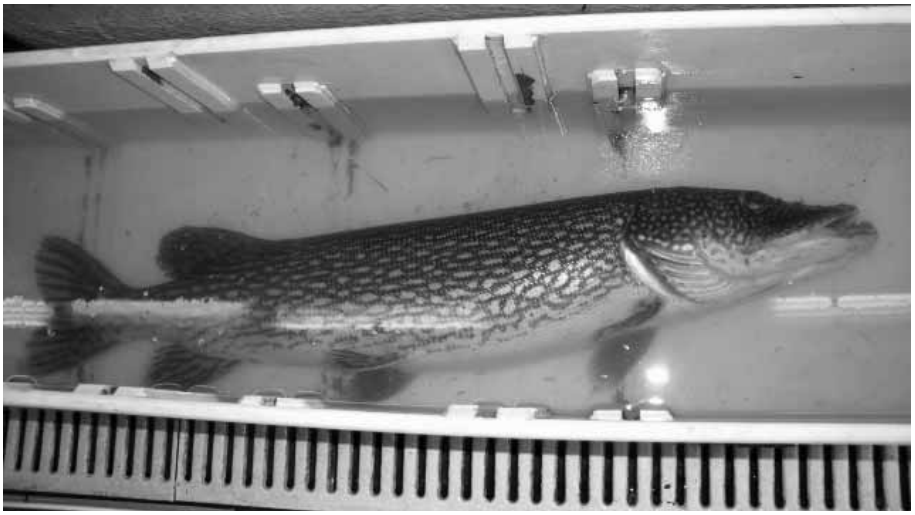

Fig. 4. *Broodstock female pike (Esox lucius L.) in anaesthesia (photo: T. Policar).*

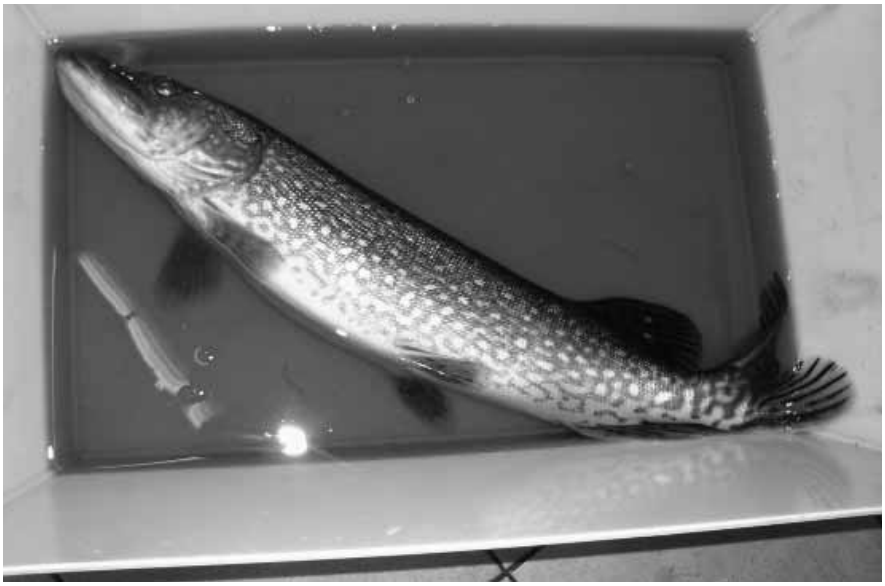

Fig. 5. *Broodstock female pike (Esox lucius L.) in a potassium permanganate bath that serves as prevention against surface fungal infections (photo: T. Policar).*

2.6. Controlled reproduction of pike by thermal and hormonal stimulation

Broodstock designated for stripping are kept in wintering ponds from autumn to spring with a sufficient amount of prey fish (smaller cyprinid fish species). It is recommended to stock one kilogram of prey fish per one kilogram of stocked pike. It is important to remove broodstock from ponds in spring period when the temperature gradually rises to 4–6 °C and a natural spawning period is approaching (Dubský, 1998). After that, broodstock must be transported either into smaller and shallow ditch ponds (surface area around 100 m^2) close to a hatchery or stocked directly into rearing troughs in a hatchery where it is possible to regulate a water temperature and thus influence the time of stripping (Policar, 2012a).

The use of the above-mentioned methods of broodstock stocking is dependent on a stripping method. If fish are stripped without a hormonal treatment, fish of both sex are stocked into ponds that are largely overgrown with macrovegetation and contain a sufficient amount of prey fish (0.5 kg of prey fish per 1 kg of pike). It is important to measure a water temperature twice a day (in the morning and in the afternoon) and to monitor behaviour of broodstock. In spring period, water gradually warms up from 4 °C (in the morning) up to 8–10 °C (afternoon) and fish behaviour changes. When temperature rises, fish stay at pond brims and they intensively swim into littoral macrovegetation. This behaviour suggests that some broodstock females are ready for spawning. After that, it is necessary to remove fish from a trench pond, select ovulating females and catch approximately the same quantity of males. Subsequently, fish ready to spawning are transferred to a fish hatchery where eggs are stripped from female by abdominal massage. After that, eggs are artificially fertilized, desticked and incubated (Policar, 2012a). Individual rearing operations are described in detail in the following chapters.

In case that broodstock are stocked under controlled conditions into fish hatchery reservoirs, hormonal preparations (Fig. 6) are applied to broodstock after initial thermal stimulation (water temperature 9–11 °C) and these preparations ensure final oocyte maturation and their

subsequent ovulation (Bondarenko et al., 2012b). This intervention must be implemented in order to prevent reproductive dysfunction in pike. (Hamáčková et al., 1975; Kouřil and Hamáčková, 1975, 1977; Mylonas and Zohar, 2001).

Fig. 6. *Hormonal injection of broodstock female pike (Esox lucius L.) (photo: T. Policar).*

2.7. Hormonal stimulation of final oocyte maturation and ovulation of eggs

As far as pike is concerned, hormonal stimulation of ovulation under controlled conditions in fish hatcheries was solved in the past mainly by hypophysation by means of dried carp pituitaries at a dose of 3–4 mg.kg^{-1} of live weight for females and 2–4 mg.kg^{-1} of live weight for males (Billard, 1996; Policar, 2012a; Švinger et al., 2012; Bondarenko et al., 2013b). This method has remained the only reliable method used in fishery practice for mass induction of ovulation for this species (Szabó, 2001, 2003, 2008). For some fish species where it was possible to replace hypophysation with application of synthetic GnRHa (Gonadotropin – Releasing Hormone analogue) or a combination of GnRHa with dopaminergic inhibitors (metoclopramide, pimozide and domperidone). In case of pike, this hormonal induction of oocyte ovulation failed either completely or its efficiency was incomparable with a success rate of hypophysation (Szabó, 2003). Billard (1996) also described a successful application of partially purified salmon gonadotropin (PPSG) in order to induce oocyte ovulation with the application of 100 μg PPSG.kg^{-1} of live weight of females. However, the above-mentioned author revealed that the efficiency of this hormonal treatment was considerably decreased in rearing of broodstock in captivity. Fish were caught and immediately hormonally treated with PPSG, 100% ovulation was achieved. In fish kept under controlled conditions for 3 days that were hormonally stimulated with PPSG after that, the ovulation rate decreased to 40%.

Problems connected with an effective induction of oocyte ovulation in pike kept under controlled conditions have recently been connected with the use of low doses of GnRHa (doses under a level of 50 μg.kg^{-1}) and a temporary non-use of a possibility offered by the

GnRHa to apply it with a prolonged effect by means of GnRHa emulsification in adjuvants (e.g., FIA – Freund's incomplete adjuvant). Therefore, in 2012, en experiment testing effectiveness of the following hormonal preparations was designed:

- sGnRHa (synthetic analogue of salmon GnRH) D- Arg^6Pro^9NEt (40–50 μg sGnRHa.kg^{-1} of live weight) in combination with the dopaminergic inhibitor metoclopramide (8–10 mg.kg^{-1}) with a subsequent emulsification in FIA,
- sGnRHa D-Arg^6Pro^9NEt (40–50 μg sGnRHa.kg^{-1} of live weight) in combination with the dopaminergic inhibitor metoclopramide (8–10 mg.kg^{-1}),
- sGnRHa D-Arg^6Pro^9NEt (40–50 μg sGnRHa.kg^{-1} of live weight) with a subsequent emulsification in FIA,
- carp pituitary dissolved in 0.9% physiological saline solution at a dose of 4 mg.kg^{-1} of live weight,
- carp pituitary dissolved in 0.9% physiological saline solution and subsequently homogenized in FIA in the rate of 1:1 at a dose of 4 mg.kg^{-1} of live weight (Policar, 2012a).

An effective hormonal intervention that induced final maturation of oocytes and ovulation of eggs in 100% of fish was carp pituitary dissolved in a physiological saline solution. Homogenized carp pituitary with FIA induced ovulation of eggs in 86.5% of fish (Bondarenko et al., 2013b). The results suggests that homogenized carp pituitary with FIA was not effective in a form of a percentage of ovulated fish in comparison to application of the carp pituitary itself. On that account, this more expensive method of hormonal stimulation of pike ovulation cannot replace in practice the used carp pituitary dissolved only in a physiological saline solution. Other hormonal treatments were ineffective (ovulation of eggs in 0% of fish) with the exception of the use of sGnRHa D-Arg^6Pro^9NEt (40–50 μg.kg^{-1} of live weight) in combination with the dopamine inhibitor metoclopramide (8–10 mg.kg^{-1}). This hormonal treatment induced ovulation in 14% of females. Nevertheless, the effectiveness of this hormonal treatment was very low and probably connected with a spontaneous ovulation. Therefore, this method of hormonal treatment cannot be recommended to be used in fishery practice either (Policar, 2012a). In addition to this experiment, the effectiveness of high doses of mGnRHa (mammalian GnRHa; doses up to 150 μg mGnRHa of Lecirelin – the Supergestran preparation.kg^{-1} of live weight) was also tested but this method did not induce egg ovulation either (Švinger, personal information, 2013).

The above-mentioned results have confirmed that the carp pituitary has remained the only effective hormonal treatment inducing oocyte ovulation. Hypophysation has been apply in hormonally controlled reproduction of fish since the 1930s when it was applied to rainbow trout (*Oncorhynchus mykiss*) for the first time (Hasler et al., 1939). A classic technique of application of dried carp pituitary is their dissolution in 0.7–0.9% physiological saline solution (NaCl) and an intramuscular or intraperitoneal single application at a dose of 3–4 mg.kg^{-1} of live weight of females or 2 mg.kg^{-1} of live weight of males (Pecha et al., 1992; Szabó, 2001, 2008).

Weighing of the required amount of pituitaries must be carried out on a corresponding laboratory weight with accuracy of at least ± 0.001 g. The pituitary is diluted with the above-mentioned physiological saline solution in such a way that a hormonal preparation is given to fish in a volume of 1 ml.kg^{-1}, i.e., with respect to females, 3–4 mg of pituitary is dissolved in 1 ml of a physiological saline solution and, as far as males are concerned, 2 mg of pituitary are dissolved in 1 ml of a physiological saline solution. In order to achieve a thorough homogenization of pituitaries and their mixing with a physiological saline solution, it is advisable to use a laboratory ceramic grinding mortar with a pestle.

2.8. Hormonal treatment of males

Smaller-sized males (0.5–1 kg) are used for artificial stripping of pike (Dubský, 1998). If pike males are hormonally stimulated, dried carp pituitary at a dose of 2–4 $mg.kg^{-1}$ of live weight is used (Policar 2012a; Švinger et al., 2012). Males are injected intramuscularly after anaesthesia (see chapter 2.5) in a period when females are hormonally treated (usually 4 days before the fish stripping itself). The decision whether to apply a hormonal treatment of males depends mainly on a method of sperm collection and on the decision of a responsible employee at a given hatchery. If it is planned to use extracted released sperm, it is advisable that males are hormonally stimulated (Policar 2012a; Švinger et al., 2012). If males are not hormonally treated, it is probable that only a very little amount of sperm around 0.5–2 ml per one male will be obtained (Billard, 1996; Dubský, 1998; Hulák et al., 2008a,b). Such small amount of sperm can cause great difficulties at artificial fertilization of obtained eggs. If so-called testicular sperm is used (sperm obtained from dead males when testicles are removed from male bodies and dissected) it is not necessary to use hormonal stimulation (Hulák et al., 2008a,b). Nevertheless, hormonal stimulation of males is recommended even for this method of sperm extraction. Application of a hormonal treatment increases production of sperm in testis. This fact facilitates the work and positively influences artificial fertilization of eggs. A sufficient amount of sperm for egg fertilization is consequently manifested in a higher rate of egg fertilization (Policar, 2012a).

2.9. Length of latency period, synchronization and a success rate of female stripping

Latency period (latency interval) is a period between hormonal stimulation and stripping of ovulated eggs (Policar et al., 2011a). Latency period of females that are treated with a dried carp pituitary at the above-mentioned dose ranges from 96.0 ± 14.4 and 98.2 ± 2.5 hours from the performed hormonal injection (Policar 2012a; Švinger et al., 2012). In some cases it has been revealed that all females treated with carp pituitary stripped at the same moment, i.e., 96 hours after the hormonal stimulation (Bondarenko et al., 2013b). When a different experiment was carried out, identically treated females were stripped at the same latency period (96 hours), however, the stripping period from the first until the last stripped female took 12 hours (Policar, 2012a). Length of latency period in day degrees ranges around 42.0 ± 6.3 °d which means that fish were kept at a temperature of 10.5 °C for 4 days between the injection and stripping (Policar, 2012a). The success rate of stripped females treated with carp pituitary amounted to 95–100% (Policar, 2012a; Švinger et al., 2012). With regard to pike females which were treated with carp pituitary mixed together with Freund's incomplete adjuvant, latency length of 107.9 ± 10.3 hours was revealed when during 12 hours all females were stripped (Bondarenko et al., 2013b). With regard to females treated with the sGnRHa in combination with metoclopramide, only 14% of treated females stripped at a latency period of 97.5 hours (Policar, 2012a).

Females stimulated only with a rising water temperature have a stripping period much longer than hormonally treated fish. In the course of several stripping periods it was discovered that broodstock stocked into the trench pond close to the fish hatchery in Nové Hrady Fisheries Ltd stripped gradually within approximately one month. For example, in 2012, these stripped fish ovulated during 30 days from 22nd March until 20th April. The success rate of the stripping, that is characterized as an amount of stripped individuals, amounted in these fish to 95% (Fig. 7 and 8). In total, 66% of fish was stripped during the first 8 days of a given stripping period (Policar, 2012a).

Fig. 7. *Ovulating broodstock female pike (Esox lucius L.) prepared for strip (photo: T. Policar).*

Fig. 8. *Stripping of broodstock female pike (Esox lucius L.) (photo: T. Policar).*

2.10. Fecundity of females

An absolute fecundity of females largely fluctuates in dependence on their size, age and a locality where pike occurs (Nikolsky, 1963; Kouřil and Hamáčková, 1975; Billard, 1996; Hubenova and Zaikov, 2007). With an increasing body weight and length, an absolute fecundity of females also increases (Billard, 1996; Hochman, 1964). Hochman (1964) presented a dependence of a number of eggs on a female size in South Moravian ponds (Tab. 1). Dependence of an absolute fecundity on age, size and weight of females was published by Billard (1996) and these values are provided in Tab. 2. Similar results were also presented by Křišťan et al. (2013) and he stated that an absolute fecundity had ranged from 65 000 to 141 000 fish eggs.

Tab. 1. *Number of eggs in individual size categories of female pike (Esox lucius L.) (Hochman, 1964).*

Body length (mm)	300-350	350-400	400-450	450-500	500-550	550-600	600-650	650-700	700-750	750-800	800-850
Absolute fecundity (thousands. pcs of eggs)	10.2	16.5	25.2	36.9	52.5	71.0	94.6	127.2	159.5	190.8	248.0

Tab. 2. *Number of eggs in individual age, size and weight categories of female pike (Esox lucius L.) (Billard, 1996).*

Age (years)	Total length (mm)	Weight (g)	Absolute fecundity (pcs x 1000)	Number of fish
2	325–560	330–2 100	6.0–42.0	23
3	410–720	700–2 900	13.0–80.0	203
4	445–830	1 040–5 340	9.0–127.0	246
5	475–850	1 151–6 500	16.0–167.0	301
6	550–900	1 700–7 200	41.0–250.0	194
7	530–910	1 700–7 600	58.0–165.0	52
8	540–890	2 100–6 200	64.0–203.0	24
9	680–1000	3 000–10 560	71.8–232.6	25
10	750–1020	4 200–10 000	99.8–233.0	13
11	870–960	6 500–9 400	147.2–188.3	4
12	920–940	7 170–7 300	178.2–178.8	2
13	900	7 700	168.8	1
14	950	7 800	126.1	1

Křišťan et al. (2013) indicated a relative fecundity (a number of eggs per a unit of weight) ranging from 20 857 to 31 887 eggs per 1 kg of live weight of fish with a mean value amounting to 26 372 pcs.kg^{-1}. Similar results were also published by Hochman (1964) who determined a relative fecundity within the range of 19 712–49 901 pcs.kg^{-1} with a mean value of 28 652 pcs.kg^{-1}.

2.11. Impact of selected factors on egg fertilization and survival of embryos during their incubation

In general, there is a great problem with regard to pike eggs and embryos that is connected with a high mortality rate both of fertilized eggs and incubated embryos. The mentioned mortality rate is manifested by a lower percentage of egg fertilization and by a low percentage of hatched larvae (Horvát, 1983; Billard, 1996; Policar, 2012a). Fertilization of eggs and hatching of larvae are influenced by several factors. The most important ones involve: stripping period of broodstock, hormonal treatment of females, ovarian plasma pH level, age of broodstock, manipulation with gametes, physiological quality of oocytes and sperm, well-conducted and optimized process of artificial fertilization and incubation of eggs (Billard, 1996; Policar, 2012a; Švinger et al., 2012). Impact of the first five mentioned factors is explained in this chapter. Quality of gametes and a process of artificial fertilization of eggs are explained in the following chapters.

With respect to broodstock that were not hormonally treated, the highest hatching rate of larvae ranging from 52 to 69% was discovered during a month-long stripping period at the beginning of the period. The lower hatching rate of larvae (12–42%) was found in the last decade of the stripping period (Policar, 2012a).

If females are hormonally treated with a dried carp pituitary, fertilization of eggs ranged from 40 to 63% and hatching of larvae was lower approximately by 7–10% than egg fertilization (Horváth, 1983; Policar, 2012a). Billard and Marcel (1980) stimulated a final maturation of oocytes and ovulation of eggs with single doses of dried salmon and carp pituitaries and the values of egg fertilization achieved very different values ranging from 8 to 63%. Szabó (2001, 2008) managed to improve egg fertilization values by means of preparations ensuring a gradual release of gonadotropin during a hormonal treatment of fish. If carp pituitary in 8% solution of sodium salt of carboxymethyl cellulose (CMC-Na) was applied, fertilization of eggs amounted to 66% as opposed to 41% achieved when a classic method of hypophysation in a physiological saline solution was employed. Similar improvement in fertilization of eggs was achieved due to application of the 2% aqueous dispersion of synthetic resin Carbopol 971 P.

A value of an ovarian plasma pH level is considered to represent one of the main indicators determining quality of obtained eggs, or more precisely, of their fertilization (Wojtczak et al., 2007; Lahnsteiner et al., 1999). A decrease in an ovarian plasma pH level can occur if more acidic content of eggs (6.47) penetrates the ovarian plasma. Such phenomenon was noticed at degradation of egg membranes during over-maturation of eggs (Lahnsteiner, 2000) or if eggs are mechanically damaged (Dietrich et al., 2007). In case of pike, the value of ovarian plasma pH was used for the first time by Švinger et al. (2012) as a factor influencing the quality of eggs before fertilization and a survival of embryos in the course of incubation up to the stage of so-called eyed eggs. In the above-mentioned experiment, average values of the ovarian plasma pH level in injected females varied between 7.68–8.39 and evidently higher values were recorded in fish where hypophyseal gonadotropin was provided in an emulsified with Freund's incomplete adjuvant (FIA). Regression analysis confirmed a slightly positive dependence of survival of embryos to the stage of eyed eggs on a higher value of an ovarian plasma pH level. Nevertheless, this relationship must be further verified as the above-mentioned experiment employed a limited number of broodstock (Policar, 2012a; Švinger et al., 2012).

Our experiments also suggested that if older (4–5 year-old) and larger females with an average weight of 4 600 ± 1 450 g are used, survival of embryos to the stage of eyed eggs can be very low (25–28%) (Švinger et al., 2012; Policar, 2012a). These values were obtained after

hormonal treatment of broodstock with a field-tested carp pituitary or without a hormonal treatment. Manipulation with gametes was careful and minimal. Artificial fertilization of eggs was conducted by a certified method. On that account, a very low survival of embryos is attributed to the old age of used broodstock. According to fishery practitioners, it is possible to observe a lower viability of pike embryos after stripping of large and old broodstock females or if collection of ovulated eggs into a grinding mortar is not conducted carefully. In case that eggs are extracted from female bodies by palpation of abdominal parts during stripping, ovulated gametes must not be placed in the bowl from great height (20 cm) (Zvonař, personal information, 2012).

We discovered that a high mortality rate in fertilized eggs and embryos during their incubation (up to 60–70%) occurred in eggs that originated from the first or last parts of an egg harvest. Therefore, during stripping of females, it is recommended to remove and not fertilize those eggs that are obtained as the first or last eggs from cranial or caudal part of ovary.

2.12. Egg size and number of eggs in 1 gram

A size of unfertilized eggs obtained from fish of various sizes and different environments ranges from 2.3 to 3 mm (Toner and Lawler, 1969) and eggs are globular in shape (Frost and Kipling, 1967). In general, younger females produce smaller eggs than older fish. One gram contains from 96 to 155 pieces of unfertilized eggs (Krupauer and Pekař, 1965; Křišťan et al., 2013). A size of eggs increases after fertilization and hydration. Eggs reach a size of 2.6–3.6 mm three hours after fertilization (Forst and Kipling, 1967).

2.13. Methods of sperm collection

As it was already mentioned in chapter 2.8, there are two methods of sperm collection that are generally used for pike (Billard, 1996). The first method involves the use of sperm released from vas deferens by palpation of abdominal region after thermal or hormonal stimulation (Billard et al., 1980; Dubský, 1998). Extracted sperm is applied either directly onto stripped eggs or, more often, is drawn into syringes or pipettes in a volume of 5–10 ml (Berka and Hamáčková, 1980; Hamáčková, 1987; Dubský, 1998; Hulák et al., 2008a). Collection of extracted sperm has an advantage consisting in preservation of sperm for further using. A disadvantage of this method is that sperm gets often contaminated with urine or blood during extracting sperm from male bodies which causes a sperm activation itself and a limited usability for artificial fertilization of eggs (Billard, 1978; Hulák et al., 2008a,b). To prevent sperm contamination with urine, Berka and Hamáčková (1980) recommended to place a male on the side during sperm collection. After that, they suggested to take a catheter that had been adapted from Pasteur capillary pipette and insert it into male genitourinary papilla. This released and discharged urine. Another disadvantage of this sperm collection method can be a very limited amount of sperm from single male. This fact can limit a successful artificial fertilization of eggs or it can require utilisation of a great amount of males which comprises a very demanding organization of work at fish hatcheries (Koldras and Moczarski, 1983; Linhart, 1984; Billard, 1996).

A second method of sperm collection is a use of testicular sperm that can be obtained from a dead male after testicles were removed from its body (Fig. 9) (Billard, 1996; Lahnsteiner et al., 1998; Hulák et al., 2008a). It is important to dry and disrupt testicles after their removal (Fig. 10). Sperm is sieved through a fine polyamide fabric called uhelon (size of meshes of 300 μm) directly onto obtained ovulated eggs (Fig. 11 and 12) (Dubský, 1998). The advantage

of this method of sperm collection in comparison to the previous method lies in obtaining a larger volume of sperm with a higher concentration of sperm per 1 ml, and, above all, the collection ensures clean uncontaminated sperm with urine or blood. The disadvantage of this collection method consists in killing a male, thus in a loss of a broodstock for further rearing (Hulák et al., 2008a). Collected testicular sperm or directly obtained whole testicles can be preserved for 24–48 hours at a temperature of 2–4 °C after drying and blood removal from their surface (Křišťan, personal information, 2013).

Fig. 9. *Removal of testes from a euthanased male pike (Esox lucius L.) (photo: J. Křišťan).*

Fig. 10. *Disruption of testes in order to obtain testicular sperm for artificial fertilization of eggs of pike (Esox lucius L.) (photo: J. Křišťan).*

Fig. 11. *Transfer of crushed testicles of pike (Esox lucius L.) onto a fine uhelon fabric (mesh size 300 μm) (photo: J. Křišťan).*

Fig. 12. *Squeezing of sperm from crushed testicles of pike (Esox lucius L.) through a fine uhelon fabric onto eggs (photo: J. Křišťan).*

2.14. Male reproductive ability and characteristics of their sperm

Concentration of sperm in testicles before stripping period varies from 2.4 to 4.1 x 10^{10} of sperm.g^{-1} in testicles. Total possible production of sperm in one male ranges from 4.4–7.9 x 10^{11} of sperm per 1 kg of live weight of fish (Billard et al., 1983). Sexually mature males release sperm from November until May and a percentage of males producing sperm in this period considerably fluctuates. In November and December, it is less than 15% of males, in January and February, a percentage of males releasing sperm rises to 25–30%. In March and April, more than 60% of males release sperm spontaneously. Contrariwise, in June, there is only a several percent of males which release sperm spontaneously (Billard, 1996).

An actual production of sperm of males which release sperm is determined mainly by a volume of obtained sperm and a concentration of sperm in 1 ml of obtained sperm. Male reproductive ability considerably fluctuates depending on a size and age of a male and also on time when sperm is collected from male. At the beginning of a stripping period, males produce a little amount of sperm ranging from 0.3 to 0.4 ml.kg^{-1} of live weight of fish. The sperm production increases to a value of 1.35 ml.kg^{-1}. At the end of a stripping period, sperm production quickly decreases (Billard, 1996). According to Krupauer and Pekař (1965), an amount of collected sperm is very low and it reaches a volume of 3 ml at maximum. In practice, a lower volume of sperm ranging from 0.5 to 2.5 ml is collected, on average, from one male (Koldras and Moczarski, 1983; Linhart, 1984; Hulák et al., 2008a).

At the beginning of a stripping period, sperm is thick and cream- to snowy white-coloured. Towards the end of this period, sperm is much thinner or even watery (Kouřil and Hamáčková, 1975). An average sperm thickness in a stripping period achieves a value of 22.26 x 10^9 of sperm.ml^{-1} in sperm, with a minimum amount of 2.5 x 10^9 and a maximum amount of 68.0 x 10^9 of sperm.ml^{-1} (Kouřil and Hamáčková, 1975; Linhart, 1984; Hulák et al., 2008a).

An absolute and relative fecundity was assessed by Linhart (1984) who revealed an absolute fecundity ranging from 3.2 to 25.2 x 10^9 of sperm per one male in 38 males (TL = 355–540 mm). A relative fecundity of males varied from 1.4 to 36 x 10^9 of sperm.kg^{-1} of live weight of a male.

Osmolality of released sperm achieves values of 204–314 mOsmol.kg^{-1}. The duration of sperm motility period is dependent on an activation solution. If sperm is activated with distilled water, the period of sperm motility will be shorter in comparison to sperm activated with urine. In general, it applies that a percentage of moving sperm quickly decreases with time. Only 60% of sperm are motile 15 seconds after activation and only 35% or 10% after 30 or 40 seconds after activation. The velocity of sperm motility 15 seconds after activation is 163 ± 40 μm (Hulák et al., 2008a,b)

Hulák et al. (2008a,b) engaged in comparison of composition of stripped and testicular sperm. The above-mentioned team of authors revealed that testicular sperm had higher concentration of sodium (Na^+ = 123 ± 9 mM), chloride (Cl^- = 127 ± 7 mM) and potassium ions (K^+ = 35 ± 5 mM), and next, it had higher osmolality (358 ± 77 mOsmol.kg^{-1}) and a higher concentration of sperm in 1 ml (34 ± 5 x 10^9.ml^{-1}) as opposed to stripped sperm where the following average ion concentration was discovered: Na^+ = 116 ± 9 mM, Cl^- = 116 ± 7 mMa K^+ = 25 ± 4 mM, osmolality: 273 ± 21 mOsmol.kg^{-1} and a concentration of sperm in 1 ml: 23 ± 4 x 10^9.ml^{-1}.

2.15. Sperm morphology and characteristics

Sperm from pike, as well as from all the other fish species, consists of three main morphological parts – head, midpiece and tail that has a typical flagellate shape. Pike sperm belong to primitive so-called "aqua" sperm (Alavi et al., 2009a,b). The size of head ranges from 1.2 μm to 1.6 μm. The shape and size of a sperm head is very important since sperm head penetrates the egg membranes of eggs, especially through the microphyle opening. The whole structure of flagellum consists of two central and nine peripheral tubules. Sperm flagellum can be divided into a proximal, central and tail part. Sperm flagellum of pike has a so-called "fin" (Alavi et al., 2009a) (Fig. 13).

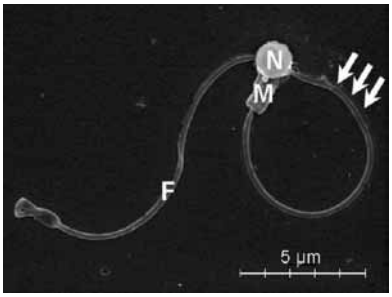

Fig. 13. *Morphology of pike (Esox lucius L.) sperm under an electron microscope (Alavi et al., 2009a); sperm head with a cell nucleus (N), midpiece (M)) and flagellum (F) with a peripheral fin (arrows) (photo: S.M.H. Alavi).*

2.16. Artificial fertilization of eggs with using of activation medium

Fertilization of eggs in a proportion of 3–4 ml of sperm to 1 kg of eggs is carried out immediately after the stripped sperm is collected. Sperm should be collected separately at least from 3 males (Billard, 1996; Křišťan et al., 2013). Křišťan et al. (2013) optimized the proportion of sperm per 1 fertilized egg and they determined the most suitable proportion of ensuring a high rate of egg fertilization (67%) and larval hatching (65%) at a level of 500 000 sperm per 1 egg, which approximately corresponds to the above-mentioned proportion of sperm and eggs during fertilization. Billard (1996) recommended the proportion to be a bit lower – 400 000 sperm per 1 egg, with the rate of egg fertilization ranging around 60–70%.

After sperm is applied onto eggs, it is advisable to gently mix sperm and eggs together. It is necessary to be very careful because eggs are highly sensitive to an excessive and thoughtless treatment at this time. Consequently, it is important to use activation medium (Fig. 14) in a volume of 0.5 l of activation medium per 1 kg of eggs over the mixture of eggs and sperm. The simplest activation medium is fresh water from a given fish hatchery. However, if artificial fertilization of eggs is carried out, it is recommended to use a activation medium of salt (NaCl) in a concentration of 7 $g.l^{-1}$ in order to achieve better and longer sperm motility. Scientific literature (Dyk, 1940; Berka and Hamáčková, 1980; Alavi et al., 2009a) also recommended using various activation medium that can increase the percentage of egg fertilization:

- Ringer's activation medium containing: 6 $g.l^{-1}$ NaCl, 0.075 $g.l^{-1}$ KCl, 0.15 $g.l^{-1}$ $CaCl_2.2H_2O$ and 0.1 $g.l^{-1}$ $NaHCO_3$ (Dyk, 1940),
- solution prepared by mixing of 15 g of urea in 1 litre of water (Berka and Hamáčková, 1980) and

- NaCl activation medium adapted by 20mM Tris to an osmotic pressure of 288 mOsmol and pH 8.5 (Alavi et al., 2009a).

An average fertilization of pike eggs in fishery facilities after artificial stripping and egg fertilization ranges around 40–70%. Egg fertilization can be considerably increased by using some of the above-mentioned activation solution to a level of up to 75–80%.

After sperm activation, the mixture of sperm, eggs and activation medium is gently mixed (Fig. 15) and is left to stand for 5–10 minutes (Billard, 1996; Policar 2012a). Then, the mixture of eggs and sperm is rinsed several times with water and fertilized eggs freed of sperm residues are thus prepared to eliminate the stickiness (so-called desticking) of eggs.

Fig. 14. *Activation of sperm with an activation medium in pike (Esox lucius L.) (photo: J. Křišťan).*

Fig. 15. *Gentle mixing of eggs, sperm and an activation medium during artificial fertilization of pike eggs (Esox lucius L.) (photo: J. Křišťan).*

2.17. Elimination of egg stickiness before incubation

Surface of fertilized eggs of pike becomes sticky in several minutes (4–5 min) after water is added. It is necessary to mention again that elimination of egg stickiness must be carried out very gently since fertilized eggs are highly sensitive to manipulation. For desticking of pike eggs, it is recommended to use several following methods that our team successfully tested in practice. Individual methods of egg desticking are classified in the text according to the easiest obtained media or chemicals used in this process. However, the easiest obtained

media or chemicals generally require a longer application use that leads to completion of egg desticking:

- application of cow milk containing 3.5% of fat that must be used for 60–90 minutes (Hamáčková, 1987),
- application of pond clay or clay used for production of ceramics (Fig. 16) for 30–40 minutes (Dubský, 1998; Policar, 2012a),
- application of a solution obtained by mixing of 100 g of talc and 20–25 g of salt (NaCl) in 1 litre of water for 20–30 minutes (Hamáčková, 1987) and
- application of a solution obtained by mixing of 5.52 g of NaCl; 3.75 g of glycine and 2.42 g of Tris in 1 litre of water for 15 minutes
- Berka and Hamáčková (1980) also recommended eliminating the egg stickiness with the use of 5% solution of powdered starch with the exposure time of 10 minutes.

The above-mentioned desticking solutions are used usually in the volume proportion of 1 : 2 (eggs : solution). After desticking of egg surface, individual eggs start to separate from each other and they do not form a mass stuck together any longer. At this moment, desticked eggs are rinsed several times with clean water from a hatchery and they are placed in incubation bottles.

Fig. 16. *Elimination of stickiness of eggs of pike (Esox lucius L.) by means of clay (photo: J. Křišťan).*

2.18. Incubation of eggs and hatching of embryos

In the majority of cases, incubation of pike eggs in Europe is conducted in Chase bottles (Fig. 17). It is necessary to keep eggs in permanent, however, only a subtle movement in incubation bottles by means of inflowing water. A "subtle movement" must be emphasised because it has been confirmed that too sharp or strong flow rate can cause an excessive movement of eggs which thus results in an increased mortality of incubating eggs and embryos. In addition to Chase bottles, it is also recommended to use modified Zug bottles in fishery practice. In adapted Zug bottles, a perforated funnel is installed at the bottom of bottles through which water inflowing from the bottom of a bottle is gently flowing through. It ensures a weak water flow rate and a subtle movement of incubated eggs. The advantage of modified Zug bottles is that a fish hatchery does not have to purchase other special types of incubation bottles and it can use common Zug bottles for egg incubation. For incubation of pike eggs, it is also recommended to use McDonnald or Kannengieter bottles that are, however, mostly used in the USA or Western Europe (Hochleithner, 2004). Water flow rate in incubation Chase bottles in a volume of 5 l is set to a level of 3–6 $l.min^{-1}$ and 4–8 $l.min^{-1}$ in ten-litre Zug bottles. A lower water flow rate in bottles is always employed at the beginning of incubation and a higher rate of water flow rate at the end of incubation.

Fig. 17. *Artificial incubation of eggs in Chase bottles (photo: T. Policar).*

In general, 2 litres of eggs are placed into ten-litre modified Zug bottles and eggs consequently become swollen and increase their volume (up to three times). After swelling up, eggs can represent up to 2/3 of volume of a incubation bottle. Oxygen content in water flowing into incubation bottles should range from 7 to 9 mg $O_2.l^{-1}$. Water used for incubation of pike eggs should be filtered through sieves of a mechanical filter and it should thus get rid of coarse impurities. Water used for pike incubation should be of a similar quality as water used in salmonid hatcheries. Billard (1996) and Hochleithner (2004) shared the same opinion.

Optimal water temperature for egg development varies between 6–10 °C (Bondarenko et al., 2013a; submitted). At the beginning of egg incubation, fishery facilities usually use a water temperature ranging from 8 to 10 °C that is gradually increased to a temperature of 12–14 °C towards the end of incubation. Hamáčková (1987) reported a high mortality rate of eggs and embryos during incubation with water temperature from 4 °C to 22 °C. Lillelund (1967) and Hokanson et al. (1973) monitored experimentally the development of pike eggs at a temperature range between 3.7–24 °C. Survival of embryos higher than 80% was achieved at a water temperature varying between 6.4–17.7 °C. When a water temperature achieved 3 °C, only 9% of larvae hatched. According to Swift (1965), the optimal incubation temperature is 9 °C. This water temperature ensured the highest hatching rate of pike embryos (60–80%). Lillelund (1967) incubated pike eggs also at temperature of 5.8 °C with a good hatching rate of larvae at a level of 70%. Nevertheless, he stated that with regard to such incubated eggs, there was a high mortality of larvae one day after hatching. The author noticed that if hatched larvae were immediately transferred after hatching to temperature of 9–18 °C, a considerably decreased mortality of larvae was achieved. It is also necessary to avoid great water temperature fluctuation during egg and embryo incubation. Daily temperature changes by 5 °C (from 15 °C to 20 °C) caused a decrease in a hatching rate by 12% (Lillelund, 1967). For the first 30–40 °d, any manipulation with eggs must be avoided (mixing or releasing eggs stuck together). Such excessive manipulation causes increased losses by 20–25%. Dead and live eggs at the stage of eyed eggs can be separated by means of so-called separating

solution of NaCl prepared by dissolving of 136 g of salt in one litre of water. Separation of dead eggs from one incubation bottle takes about 1 minute. Live embryos float on the surface which must be transferred back to fresh water in a newly prepared incubation bottle as soon as possible. Removal of dead eggs is advisable to be carried out to protect live incubated eggs against fungal infection (most frequently the *Saprolegnia* and *Achlya* genera). In addition to removal of dead eggs, it is also possible to carry out a short immersion antifungal bath in salt (NaCl) in a concentration of 20 $g.l^{-1}$ for 20 minutes without a water flow at the half of the egg incubation period (approximately 5–8 days after fertilization). This bath can be carried out directly in an incubation bottle or outside the bottle.

At the eye-stage or at the beginning of the hatching period, it is advisable to transfer embryos into flat hatchery apparatuses or cradles with a mesh size of 2–5 mm at their bottoms or walls for so-called final hatching (Fig. 18). At this time, it is necessary to stop the inflow of water into incubation bottles. Subsequently, embryos are gently removed by suction without strong shaking movements into buckets by means of which the removed embryos are placed into the above-mentioned apparatuses or cradles.

Total incubation period of pike is dependent on a water temperature. If a water temperature varies between 8–10 °C, incubation lasts 110–140 °d which is between 11 to 17.5 days. If the temperature is 14 °C, it is 85 °d (6 days) and if the temperature reaches 18 °C, the sum of daily degrees required for larval hatching is 61 °d (3.42 days) (Bondarenko et al., 2013a, submitted). The incubation period of pike with the use of various water temperatures was published by Lillelund (1967) and individual values are provided in Tab. 3.

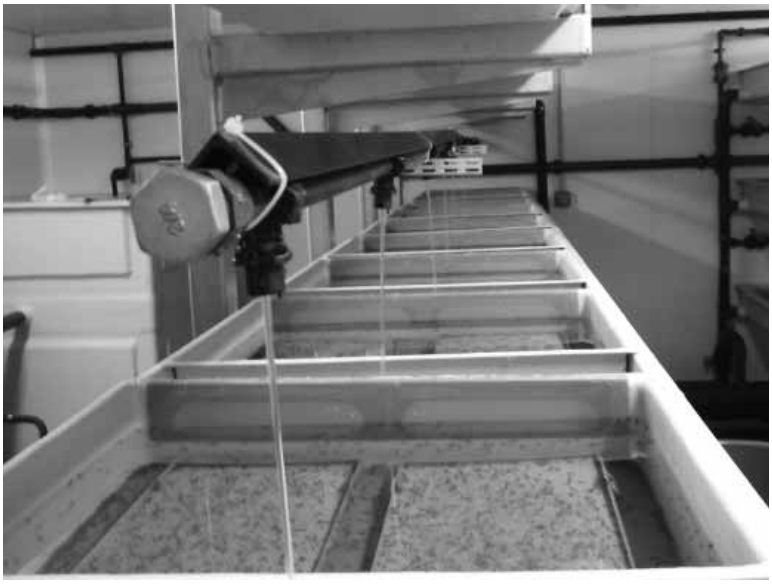

Fig. 18. *Final hatching of pike larvae (Esox lucius L.) on flat hatching apparatuses (photo: J. Křišťan).*

Tab. 3. *Length of incubation period depending on a water temperature during incubation of eggs and embryos of pike (Esox lucius L.) (Lillelund, 1967).*

Water temperature (°C)	Length of incubation	
	days	°d
5.8	30.9	179
9	15.2	137
12	9.4	113
15	6.3	95
18	4.7	85

The length of the process of larval hatching itself is also largely dependent on a water temperature. Based on our experience, this period can last 4 days at a water temperature of 6 °C and less than a day (0.92 of a day) at a water temperature of 18 °C (Bondarenko et al., submitted). Larval hatching can be accelerated by a steep increase in a water temperature by 5–7 °C which decreases the concentration of dissolved oxygen in water. Such increase in a water temperature is advisable to be carried out at a time when approximately 10% of larvae have already hatched. In the course of the intensive hatching of embryos, it is highly important to remove egg skin and dead embryos by suction at regular intervals of 2 to 6 hours.

2.19. Rearing of larvae until the yolk-sac resorption

An average size of newly hatched larvae of pike ranges from 8.5–9 mm with an average weight of 10–11 mg (Billard, 1996). It is advisable to provide pike larvae in hatching apparatuses, cradles or flat troughs with a possibility to hang on a substrate in a form of straw, branches of conifers, birches or a plastic netting (Fig. 19) with a mesh size of 1–5 mm immediately after hatching (Hamáčková, 1987; Billard, 1996). Larvae start hanging vertically by means of an adhesive papilla that develops on their head within several hours after hatching. Larvae stay in this position for 8–16 days depending on a water temperature which can vary in ponds between 8–16 °C. Hanging of pike larvae on a substrate takes approximately 130 °d. An adhesive papilla starts to be absorbed usually on the 9th day after hatching at a water temperature of 14 °C (Billard, 1996). A substrate enabling larval hanging is not necessary for maintaining the hatched larvae, however, it is suitable because it ensures a very good larval survival (Westers, 1986). After larvae start swimming, they swim towards the water surface and their swim bladder fills in. After that, larvae swim on the water surface or in water column and they digest a yolk-sac. Larvae have a fully functional mouth the second to fourth day after hatching and anus is fully developed within fourth to fifth day after hatching (Balvay, 1983). A complete resorption of a yolk-sac is terminated within 160–180 °d after larval hatching. Larvae start to take in the first exogenous food even before a complete digestion of a yolk-sac approximately 150–160 °d after their hatching. At this time, their size is around 12–15 mm and weight is 12 mg. At this stage, pike larvae swim actively and they quickly start foraging for food (Luquet and Luquet, 1983). Pike larvae are recommended to be stocked into further rearing optimally at the period when more than 50% of larvae keep hanging and other fish swim actively and hang only occasionally. Transport and stocking of larvae into rearing can still be carried out the following day when the majority of fish has already started to swim and only 25% of fish keep hanging. Later larvae stocking is not advisable because larvae starve which results in high losses within several days.

Fig. 19. *Larvae of pike (Esox lucius L.) after they started to swim in a vertical apparatus with a plastic netting used for their hanging (photo: T. Policar).*

2.20. Transport of larvae and juvenile pike intended for further rearing

Larvae intended for further rearing must usually be transported to far distances and the transport duration may last up to 12 hours. In such a case, it is recommended to transport larvae in polyethylene bags with oxygen atmosphere. In a bag with a volume of 20 litres containing 10 litres of water and 10 litres of oxygen atmosphere, up to 50 000 pcs of hanging larvae or of those that have partially started to swim can be transported at a water temperature of 10–12 °C. In bags without oxygen atmosphere, only 30–800 pcs larvae.l^{-1} at a water temperature of 12 °C should be transported and transport duration should not exceed 3 hours.

A similar method of transportation can also be applied to reared advanced pike fry (Fig. 20). It is recommended to transport 300–500 pcs of pike in one polyethylene bag in a volume of 10 litres of water and 20 litres of oxygen atmosphere for the duration of 6 hours at maximum. Since severe pike cannibalism occurs during the transport, it is advised to transport pike in total darkness. Even more effective transport method of a greater amount of pike (20–30 thousand pieces) is a packing transport box of a usable volume of 1 000 litres that is placed on a vehicle.

Fig. 20. *Advanced pike fry (Esox lucius L.) prepared for transport – packed in polyethylene bags with water and oxygen atmosphere (photo: T. Policar).*

2.21. Possibilities of rearing of larvae and juvenile pike to the advanced fry

After larvae have successfully started swimming and have partially digested a yolk-sac, it is necessary to initiate immediately their rearing. In the Czech climatic conditions, natural rearing of pike larvae starts around mid-March and lasts until mid-April. In connection with an intensive rearing of pike in recirculating aquaculture systems (RAS), off-season stripping of broodstock has currently also started to be carried out. It is targeted at continuous production of gametes and subsequently larvae during the entire year when pike is often stripped outside its natural reproduction period. This rearing procedure enables a better use of a rearing space of a given RAS (Muscalu-Nagy et al., 2011).

At present, there are, in total, three methods of effective rearing of pike larvae and juvenile fish to the stage of so-called advanced fry of a total length of 30–50 mm at the age of 14–25 days. Afterwards, fish are stocked into open waters or other production rearing that either uses production ponds with polycultural fish stocks or RAS for intensive rearing. All three methods of rearing of advanced fry employ rearing of pike in monoculture stocks. The first method is rearing in suitable stock-ponds, ditch ponds or ground ponds (Hamáčková et al., 1977; Berka and Hamáčková, 1980; Lusk and Krčál, 1982; Hamáčková, 1987; Dubský, 1998; Policar, 2012a). Other method is pike rearing in special rearing facilities (various cradles, storage ponds, troughs and tanks) although this rearing must be supplied with artificially caught live feed – mostly zooplankton on a daily basis (Hamáčková et al., 1977; Berka and Hamáčková, 1980; Lusk and Krčál, 1982; Hamáčková, 1987; Dubský, 1998). The last method is rearing of pike under controlled conditions of RAS where, at first, larvae are adapted to an intake of artificial pellet feed, established artificial light regime and ensured optimal water temperature for pike growth (25–28 °C) (Szczepkowski, 2009; Policar 2012b; Dušek, 2013).

2.21.1. Classic pond rearing of larvae and juvenile fish to the advanced fry

Pond rearing of larvae employs, above all, stocking into trench and ground ponds (Fig. 21) of a small area from 100 m^2 (Lusk and Krčál, 1982; Hamáčková, 1987), however, ponds of larger areas (up to 1.5 ha) can be used as well (Policar, 2012a). The ideal conditions are if it is possible to use also wintering ponds for this type of rearing that were drained over winter period before pike rearing. In general, it applies that it must be easy to remove fish from a pond and, if possible, ponds must have flat and well-sloped bottoms. It is optimal when fish can be caught at the end of rearing under the dam which enables careful removal of reared juvenile fish from a pond. Inflow and outflow from a pond must be secured against fish escape as well as against penetration of piscivorous fish species into the pond. At the beginning of pike rearing, the water surface is maintained at a level of 50 to 70 cm and after one week, it is elevated to 100–120 cm. Lower water surface at the beginning of rearing enables faster heating of pond water which positively influences the development of a pond food base in a form of zooplankton.

Ponds selected for rearing must be properly prepared for stocking of larvae itself. It is advisable to fertilize ponds of a lower trophy (ponds with a sandy bottom, a low content of phosphorus and nitrogen in their sediment and with an oligotrophic inflow) with manure (dung or compost) at a dose of 300–500 $kg.ha^{-1}$ already several days (14–21 days) before the stocking of larvae itself. When fertilization is carried out, it is very effective to spread manure on the pond bottom in a littoral zone in a form of small heaps, so-called planktonic nests (Dubský, 1998). Such fertilized ponds are subsequently filled up with water at least 12–14 days before stocking of larvae itself. This time is important to ensure a sufficient development of

zooplankton in ponds before larval stocking. Larvae which have started to swim containing the rest of a yolk-sac should be stocked into ponds with an adequate representation of moderately large zooplankton (*Diaphanosoma, Eurycercus, Daphnia, Cyclops*, etc.) at a density of 150–300 individuals.l^{-1} (Hamáčková, 1987).

Fig. 21. *Pond used for rearing of advanced pike fry (Esox lucius L.) (photo: T. Policar).*

Initial density of stocked larvae is determined according to an amount of natural food in a pond, according to a division and a length of a bank line and occurrence of aquatic vegetation in a given pond (Lusk and Krčál, 1982). In general, it applies that the higher the zooplankton density, the more indented bank parts and larger representation of aquatic macrophytes in a pond, the larger the the density of larvae can be used for stocking (Berka and Hamáčková, 1980). Based on our experience of one month's rearing, we recommend carrying out the stocking at an initial larval density ranging from 8 to 30 pcs.m^{-2} which represents a density of 80–300 thousands of larvae.ha^{-1} depending on a trophy and a size of used ponds. Smaller ponds of an area of 0.1–0.2 ha can be stocked at an initial density of up to 300 thousands of larvae.ha^{-1}. Lower density of larvae (80–100 thousands of larvae.ha^{-1}) is used in larger ponds with an area of 1.2–1.5 ha. Other pike breeders recommend the following different initial larval densities. Huet (1976) recommended to apply an initial larval density at a level of 10 000–20 000 pcs.ha^{-1} for a 6–8 week-long rearing. Steffens (1976) described rearing of advanced fry at initial larval densities of 30 000–800 000 pcs.ha^{-1}. Lepič (personal information, 2012) used small ponds of an area of 0.08–0.3 ha with an initial density of 230–250 thousands of larvae per 1 hectare. Lepič (personal information, 2012) further added that at the end of rearing, it was necessary to provide pike in ponds with food in a quantity of approximately 3–5 kg of caught zooplankton on a daily basis. Berka and Hamáčková (1980) documented the development of food in reared larvae and juvenile fish in the course of pike rearing in detail. Based on our knowledge, fish of a total length, TL = 10–12 mm eat mainly medium-sized zooplankton (*Diaphanosoma, Eurycerucus, Daphnia* and *Cyclops*), fish of a total length of 12–20 mm specialized in zooplankton and Chironomidae larvae. Fish of a total length of

20–50 mm eat food selectively in such a way that with an increasing body length fish preferred larger food organisms, such as zooplankton (*Daphnia* and benthic organisms, e.g., larvae of *Chironomidae, Trichoptera, Ephemeroptera* and *Diptera*). With regard to fish of a total length of 25–30 mm, the first signs of cannibalism appeared. Pike is reared from its larval stage until the advanced fry in ponds under a water temperature at the beginning of rearing of usually 12–13 °C and at the end of rearing, it is 14–16 °C. It must be stated that this temperature is not optimal for pike growth and it rather represents a limiting factor for the growth. Due to numerous experiments carried out under controlled conditions, it was revealed that an optimal temperature for a growth of larval and juvenile stages of pike was a water temperature ranging between 24–28 °C (Szczepkowski, 2009; Policar, 2012b). Nevertheless, the above-mentioned natural water temperature in ponds ensured a very good and economically advantageous production of advanced fry. With regard to this rearing, if balanced and sufficient density of food organisms was ensured, reared fish achieved a very high growth velocity (a specific growth rate = SGR = 22.5–30.0%.d^{-1}) and the total fish production was not affected by an excessive cannibalism rate (only 10–15 %) (Policar, 2012b).

In order to achieve a successful rearing of advanced pike fry, it is highly important to regularly monitor the density of zooplankton occurring in rearing ponds. If there is an insufficient amount of food when the zooplankton density decreases below 100 individuals per 1 litre of water, ponds can be provided with zooplankton caught somewhere else or occurrence of zooplankton can be supported by regular pond fertilization with artificial fertilizers (urea or ammonium sulphate at a dose of 20 kg.ha^{-1}). However, such fertilization of ponds must be conducted circumspectly and only in exceptional cases if occurrence of food organisms cannot be supported in any other way. Rearing of advanced pike fry must be terminated at the moment when there are fish of catchable sizes (minimum TL = 30 mm), the maximum rearing capacity is used and a decrease in coarse zooplankton density below the above-mentioned density of 100 individuals per 1 litre of water starts to occur. If the harvest of a pond is performed too late (there is a delay of only several days), it is possible to encounter a high cannibalism rate during advanced pike fry rearing and a low survival of reared fish ranging from 5 to 10%. If the rearing of advanced pike fry is well-conducted, the survival rate varies between 20 to 40%.

The following data provide results and efficiency of advanced pike fry rearing revealed by other authors under different specific conditions. If ponds are stocked with an initial density of 80 000 pcs.ha^{-1}, it is possible to achieve a final production of advanced fry in an amount of 28 000–40 000 pcs of fish per 1 ha of an area with a biomass of 23–25 kg.ha^{-1} at a survival rate of 35–50% in 14–17 days of rearing (Berka and Hamáčková, 1980). Survival of juvenile pike with a final total length of 90–100 mm after a six to eight-week long rearing at an initial density of 10 000–20 000 larvae achieved the level of 5 to 50% (Huet, 1976). Pike cannibalism was relatively high (25–70%) during this rearing. During a twenty-day long rearing of advanced fry at an initial larval density of 300 000 pcs.ha^{-1}, only 12% of fish survived (Steffens, 1976; 1986). Their final TL reached 44 mm and individual weight was 1.2 g. Lepič (personal information, 2012) achieved a fish survival ranging from 10 to 30% during a 15–30-day long rearing. Survival was dependent mainly on cannibalism among reared fish and a final length of produced fish. Lepič added that the longer the rearing, the lower the percentage of fish of larger body sizes (TL = 40–60 mm). Policar (2012a) revealed survival of advanced fry at a level of 15–30% after 15-day long rearing of fish at a final TL of 40–45 mm. A higher survival rate was achieved in ponds at a smaller area (0.16 ha) as opposed to ponds at a higher area (1.1–1.5 ha).

The most suitable period for pond harvesting during rearing of advanced pike fry is the moment when advanced pike fry achieve a total length of 30–40 mm. The term of harvest

may be slightly postponed if a sufficient density of zooplankton and macrozoobenthos is established in a given pond. In such a case, however, zooplankton density must be carefully monitored and recorded at regular daily intervals. If a decrease in density of food organisms is discovered, harvest of reared fish must be immediately conducted. In general, it is important to catch advanced fry into underlying nets or various fishing cages under a pond dam in a very careful manner (Fig. 22). In order to catch advanced pike fry, the same general rules as for catching of advanced pikeperch (*Sander lucioperca L.*) fry are valid. The harvest should be carried out quickly (within 6–12 hours) in a suitable cloudy or rainy weather at air temperature of 18–20 °C at maximum. Fish should be regularly removed from nets and carefully sorted (Policar et al., 2011b).

Reared and caught advanced pike fry are subsequently stocked into stock-ponds or main ponds to one or two-year-old carp stock. The objective in these ponds is to minimize the occurrence of less valuable commercial species and increase the production of carp. Next, caught advanced pike fry can also be sold to sport fishermen who stock the pike fry into sport fishing grounds in open waters (Lusk and Krčál, 1982; Hamáčková, 1987; Dubský, 1998).

Fig. 22. *Harvest of pike advanced fry (Esox lucius L.) under a pond dam (photo: T. Policar).*

2.21.2. Rearing of larvae and juvenile fish to the advanced fry in special rearing facilities of a flow-through type

Rearing of advanced pike fry in special rearing flow-through facilities was mainly employed between 1970s–1990s in Germany, the Netherlands, Hungary, France, Switzerland, Austria and the former Czechoslovakia (Hamáčková et al., 1977). At present, based on our information, this system has not been used too often and in the first decade of the 21st century, it was replaced by rearing of pike in RAS (see chapter 2.21.3). Rearing of pike in special rearing facilities of a flow-through type was generally implemented from the larval stage for 2–4 weeks in different facilities, such as cradles, storage ponds, troughs and tanks (Berka and Hamáčková, 1980; Hamáčková, 1987). In these facilities, reared pike was dependent on a food supply in a form of caught zooplankton (Lusk and Krčál, 1982). Main limiting factors of this rearing were: ensuring a food base for reared fish, low water temperature (13–15 °C) and protection of fish against unicellular parasites (Berka and Hamáčková, 1980). Some farms situated in Germany,

Hungary and France employed a partial heating of a rearing system by geothermal water or waste water which enabled a stable temperature ranging between 16 and 20 °C. An increased water temperature had a positive impact on pike growth, however, there was a greater risk of cannibalism that must have been eliminated by a sufficient feeding (Hamáčková et al., 1977).

Feeding was provided in a form of live and pre-caught zooplankton, e.g., cyclops (Copepoda) and cladocerans (Cladocera). A daily feeding ration of zooplankton amounted to 25–35% of reared fish biomass (Hamáčková et al., 1977; Hamáčková, 1987). Larvae were stocked into rearing systems at an initial density of 6–8 up to 20 larvae.l^{-1} (Dubský, 1998) and they were kept at a light intensity of 250–270 lux (Hamáčková et al., 1977).

Different rearings resulted in different fish production which is summarized in Tab. 4 (Hamáčková et al., 1977).

Tab. 4. *Water temperature and production indicators within pike rearing in so-called special rearing facilities of a flow-through type (Hamáčková et al., 1977).*

Used water temperature (°C)	Duration of rearing (days)	Fish survival rate (%)	TL of reared fish (mm)
16–17	10 - 14	50	22–30
15–20	18 - 23	50	23
11	42	20	25–30

2.21.3. Intensive rearing of larvae and juvenile fish to the advanced fry in RAS

An intensive rearing employing the RAS system (Fig. 23 and 24) has been tested and experimentally used for production of juvenile pike to the advanced fry of a total length of 30–50 mm in the past 10 years mainly in Central Europe (Poland, Hungary and the Czech Republic) (Wolnicki et al., 1997; Myszkowski et al., 1998; Kucska et al., 2005; Szczepkowski, 2009; Policar, 2012b; Dušek, 2013). This system of pike rearing has enabled to use artificial pellet feed, larger densities and high growth rate of reared fish and, at the same time, to eliminate the cannibalism rate (Szczepkowski, 2009; Policar, 2012b). The objective of this pike rearing is to obtain a high-quality pike stocking material within a very limited rearing space without the need to catch and utilize zooplankton. This system of pike rearing has currently experienced the beginnings of its use. However, in Hungary, there are already three commercial fish farms (Aranyponty, Bajcshal and Szegedfish) employing this method of intensive pike rearing to the advanced fry for their production (Kucska, personal information, 2013). However, it can be expected that this system of pike rearing will be used to a greater extent in future. If combined with off-seasonal fish stripping, the system offers a possibility to produce fish continually in the course of the entire year with a high labour productivity (Policar, 2012b). Next, it was revealed that pike adapted to artificial feeding and RAS in older age categories can be effectively and successfully reared in combination with intensive rearing of sturgeons, specifically with Siberian sturgeon (*Acipenser baerii*) (Szczepkowski, personal information, 2010). The advantage of this system is a predictable fish production as opposed to fish rearing in ponds (Policar, 2012b).

Fig. 23. *Recirculating aquaculture system used for intensive rearing of pike (Esox lucius L.) to the advanced fry (photo: T. Policar).*

With regard to intensive rearing of pike, it was discovered that it was possible to successfully use a high initial larval density (20–40 larvae.l^{-1}) (Bondarenko et al., 2012a; Policar, 2012b). An optimal water temperature ensuring a high growth and survival of pike ranges between 24–28 °C, however, it is also possible to use a water temperature of only 19–20 °C (Policar, 2012b). A suitable light regime in the course of one day (24 hours) is either an uninterrupted lighting or a light regime with two eight-hour long lighting sections that are interrupted by two four-hour long sections of dark (L8:D4:L8:D4). The third regime can also be successfully used, that consists of a 16-hour long lighting section and an eight-hour long section of dark. All lighting regimes should be of an intensity of 5–30 lux (Szczepkowski, 2009; Policar, 2012b).

Fig. 24. *Rearing of juvenile pike (Esox lucius L.) to the advanced fry in a tank within the RAS system (photo: T. Policar).*

Feed produced by the BioMar Company – Larviva Wean-Ex 300 and 500 or Inicio Plus G represent a suitable feeding for intensive rearing of pike that was personally tested by our team. Larvae at the beginning of rearing until the advanced fry (Fig. 25) can be fed with granular feed of a particle size of 0.15–0.6 mm with an initial daily feeding ration of 20% of reared pike weight and a final 10% of reared fish biomass (Policar, 2012b).

Fig. 25. *Reared advanced pike fry after a thirteen-day period of rearing within the intensive rearing in RAS system (photo: T. Policar).*

After 13 days of rearing of larvae and juvenile fish in intensive conditions, it is possible to achieve the stage of advanced fry (at a total length of 28.4–30.6 mm and a body weight of 0.095–0.14 g) and a cumulative survival at a level of 54–69%. Under such rearing conditions, reared pike reach the SGR of 18–20%.d^{-1} and the cannibalism rate amounts to 15–18%. It can be stated that such pike rearing under controlled conditions of the RAS system can be interesting and effective from an economical point of view. In 13 days, it is possible to rear up to 25 000 pcs of advanced pike fry under a very small rearing system with a total volume of 8 000 litres (Policar, 2012b).

III. Comparison of the "novelty of procedures"

In the Czech literature sources, it is possible to find a scientific publication describing optimization of reproduction and rearing of pike that originated already in 1987 (Hamáčková, J., 1987. Rearing of pike. Technical standard 46 6836. Prague, The office for Standards and Measurements, 1987, 12p). It means that over the period of approximately 26 years, Czech professionals were not offered any similar scientific publication. However, in the past 26 years, knowledge on reproduction and rearing of this very interesting and commercially used fish species has considerably changed and developed. For that reason, the presented publication provides a summary of a wide range of new information concerning controlled reproduction, sperm biology and physiology, optimization of artificial fertilization, desticking and incubation of eggs and also effective rearing of larvae and juvenile fish to the stage of advanced fry.

IV. Description of application of the certified methodology

The presented methodology is, above all, intended for practical use of described information related to biology, reproduction and rearing of pike in fishery facilities in the Czech Republic. The certified methodology will primarily be applied in the Nové Hrady Fisheries Ltd. It is supposed that this methodology will support an increased production of larvae or advanced fry of pike in the future.

V. Economic aspects

Implementation of the procedures presented in this methodology into the fishery practice is not connected with high costs. The basis lies in implementation of technological measures in practice that are related to reproduction and rearing of pike (e.g., use of hormonal stimulation of broodstock with carp pituitary, optimization of sperm collection and subsequently the whole process of artificial fertilization of obtained ovulated eggs, innovation of incubation of fertilized eggs with the use of Chase bottles or modified Zug bottles, ensuring a high hatching rate and successful final hatching of larvae and subsequent optimization of rearing of larvae and juvenile fish in ponds or RAS). This activity can result in an annual higher synchronization of broodstock stripping in fishery facilities. This can save wage costs of staff (up to CZK 5 000) during the stripping period of pike and it is supposed that savings of up to several tens of hours (20 hours) required for control of broodstock without hormonal stimulation will be achieved. Next, an increase in egg fertilization and larval hatching rate by approximately 10–15% can be achieved. With respect to annual 1–3 million production of pike larvae, the production of larvae in a fishery facility can increase by 100 000–400 000 pcs. This effect can ensure an annual rise in sales by CZK 6 000–36 000 to a fishery facility with the above-mentioned yearly production and a price of pike larvae (CZK 60 for 1 000 pcs). Further application of scientific information contained in this methodology in practice can cause an increase in survival of reared advanced pike fry by 5–20% which can increase sales of a fishery facility producing yearly 100 000 pcs of advanced pike fry of a total length of 30–50 mm from this increased production at a level of CZK 22 500–90 000 every year at a price of an advanced fry of CZK 4.5 per one piece of a total length of 30–50 mm.

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