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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1. INTRODUCTION

1.1. Role of pikeperch in aquaculture

Fish and fish products have an important role in human nutrition as they are one of the essential sources of proteins, fats and amino-acids (Kristinsson & Rasco, 2000; Shalaby, 1996). In 2015, fish accounted about 17 % of total animal proteins consumed by entire human population (FAO, 2018). The demand in fish products is increasing with expansion of human population and global economic development (West et al. 2019). To fulfil market requirements, increased resource extraction from nature waterbodies has created a huge risks of overfishing of wild stocks worldwide and in some cases an extinction of particular species (Pauly et al. 2002; Myers and Worm 2003; Allan et al. 2005; West et al. 2019). In currents situation when fisheries in open waters and natural waterbodies are not able to provide a stable aquaculture production in terms of quantity and time, fish farming became an alternative solution helping to keep a growing aquatic production (Duarte et al., 2007; Fontaine et al., 2009). Implementation of a partial or full human involvement in aquaculture (e. g. control of stocking, supplementary feeding and veterinary practices) resulted in more predictable and stable supply compare to catches from wild (Policar et al., 2016). However, sustainable development of aquaculture sector requires a continuous scientific implementations and improvement of existing fisheries technics and practices. Fish reproductive biology is among the most important part of biological sciences providing a general knowledge needed for breeding in captivity (Baekelandt et al., 2018; Milla et al., 2009; Teletchea et al., 2009; Wang et al., 2010). Research and innovations forwarded to reproduction biology could extend current borders and limitations for commercial aquaculture (Baekelandt et al., 2018; Fontaine et al., 2009; Nebeský et al., 2016).

Among the percidae family, pikeperch (*Sander lucioperca*), perch (*Perca fluviatilis*), and the North American species yellow perch (*Perca flavescens*), and walleye (*Sander vitreum*), have been considered as a suitable candidates for intensive aquaculture (Ljunggren et al., 2003; Policar et al., 2016). Namely pikeperch is one of the most commercially valuable freshwater fish species in European aquaculture due to the delicate flesh and attractiveness for anglers (Křištian et a., 2013; Schulz et al., 2007). It is one of the important commercial species for temperate freshwater aquaculture, moreover, a key predator of many freshwater systems (Turesson and Brönmark 2004; Adámek and Opacak 2005; Specziár 2011). (Policar et al., 2016; Blecha et al., 2016). Wild population of pikeperch in Europe were negatively affected by industrial fishing. Such decline on wild populations and the increasing investment on commercial production of pikeperch, requires further developments on reproduction technics (Policar et al. 2016). Thus, improved fishery management, control of reproduction, effective and stable production of high-quality stocking material of pikeperch become necessary for sustainable growth of production.

Adequate broodstock management has a special importance when wild or pond-cultured is used for spawning in captivity (Migaud et al. 2013). Despite the efforts involved into development of reproduction protocols, some ecological and ethological features of pikeperch reproduction are still unknown (Lappalainen et al., 2003). Their detailed investigation and subsequent implementation into aquaculture practices could be a significant contribution to pikeperch culture development, particularly to the reproduction of wild and pond-cultured pikeperch in captivity.

1.2. Pikeperch reproduction biology

Pikeperch inhabits a wide geographic distribution, including the Aral, Azov, Baltic, Black, and Caspian Sea basins, and favors both fresh and brackish waters (Kafemann et al. 2000; Lappalainen et al. 2003; Haponski and Stepien 2013).

Pikeperch is clustered in the "early-spring spawners with paternal care" group and belongs to guarding and nest-spawning phytophils (Teletchea et al. 2009a). According to Wang et al. (2010) three main phases are required to complete reproductive cycle: (1) decreasing of temperature and photoperiod to induce a gametogenesis, (2) chilling period for vitellogenesis and (3) increase of temperature and light duration to reach final stages of oocyte maturation and induce spawning. Photoperiod and temperature are suspected to be the only two environmental factors which have influence on maturation in temperate areas fish populations (Migaud et al., 2010). Therefore, their effect is strictly implemented in percids reproduction cycle (Fontaine et al., 2015). Pikeperch gonads maturation generally completed by mid-winter (Zakęs and Szczepkowski 2004; Zakeś 2007). Nonetheless, depending on the population, reproductive traits may vary (Saulamo et al. 2005; Lehtonen et al. 2006; Olin et al. 2018). Freshwater pikeperch of the same latitude spawn earlier than individuals living in brackish water (Lehtonen et al. 2006). Habitat parameters within the population location also influence fecundity (Lappalainen et al. 2003; Saulamo et al. 2005). Pikeperch that have spent their whole lifetime in brackish water show higher fecundity than freshwater ones (Lehtonen et al. 2006).

Pikeperch exhibit strong spawning site fidelity. Spawning migration usually starts a month prior the spawning period and triggered towards the spawning ground by both salinity and temperature gradients for brackish water populations and by temperature for freshwater populations (Kafemann et al. 2000; Lappalainen et al. 2003). The homing behavior of pikeperch has been confirmed by tagging experiments (Lappalainen et al. 2003; Saulamo et al. 2005) and high genetic variability between fish groups on closely situated spawning grounds (Lappalainen et al. 2003). In general, distances in spawning migrations are shorter than 35 km, although in brackish waters some pikeperch were covering 250 km distances (Kafemann et al. 2000). Spawning areas are located in bays and river inlets where water is shallow and warms up faster where fish migrates from deep and cold wintering areas (Lehtonen et al. 1996; Olin et al. 2018). For populations occurring in brackish waters migrations forwarded to river inlets with salinity low enough to assure normal development of the eggs (Kafemann et al. 2000).

The beginning and the intensity of spawning is mainly affected by water temperature changes (Hokanson 1977; Hermelink et al. 2011, 2013). Although, according to Raikova-Petrova & Živkov (1998), spawning in nature conditions can start even at 3–4 °C. The highest temperatures are found towards the end of spawning period, up to 24 °C. However, most often the temperature range of 8–16 °C has been noted (Lappalainen et al. 2003; Migaud et al. 2010; Hermelink et al. 2013; Sarameh et al. 2013). In nature duration of spawning period also depends on the water temperature and number of age groups participating in the spawning (Lappalainen et al. 2003). As bigger difference between age groups, as longer the duration of spawning. In a warm weather, the mass spawning lasts approximately 10 days, but if temperature suddenly drops spawning may be interrupted and/or completely stopped (Lappalainen et al. 2003; Saulamo et al. 2005).

Pikeperch spawn in pairs with the males selecting and cleaning the spawning site from mud and sediments and guarding the nest until larvae are hatched (Lappalainen et al. 2003; Lehtonen et al. 2006). In the wild, pikeperch prefer dense structures such as plant roots and branches or sometimes gravel or sand (Lappalainen et al., 2003; Lehtonen et al., 2006).

Descriptions of natural spawning sites are generally vague references to "roots, branches, and plants" with size about 500 mm in a diameter and 50–100 mm thick (Lappalainen et al., 2003; Lehtonen et al., 2006; Zakęś & Demska-Zakęś, 2009). Despite wide practical application of nest spawning during reproduction in captivity, the mechanisms of spawning substrate selection in pikeperch received lack of the scientific attention.

1.3. Pikeperch reproduction in captivity

1.3.1. Origin of the broodstock

Pikeperch broodstock may be obtained from various environments. Main sources of origin are: nature water bodies, pond culture or RAS (recirculating aquaculture system). Wild fish are collected from natural environments such as lakes, rivers, lagoons (Rónyai 2007; Zakęś and Demska-Zakęś 2009; Ljubobratović et al. 2017). Spawners from natural waters are usually caught in autumn (October–November) or spring (March–May), during spawning migrations (Zakęś and Demska-Zakęś 2009). The fish caught in autumn are held for the winter in earthen ponds with forage fish (Wang et al. 2009). Pond cultured pikeperch broodstock are usually collected during autumn and spring harvesting of polyculture ponds where pikeperch is grown as a supplementary fish species (Blecha et al. 2016; Policar et al. 2016). Broodstock originated from intensive farming may be reared completely under controlled conditions of RAS from a larvae stage (RAS-cultured; Khendek et al. 2018) or in combination with pond (Rónyai 2007; Zakęś 2007; Ljubobratović et al. 2017). Depending on the origin, broodstock exhibit different levels of stress sensitivity during reproduction. Variability in stress response, production of steroids, gonadosomatic index (GSI) and quality of the gametes is significantly lower in RAS reared compare to wild and pond-cultured pikeperch broodstock (Rónyai 2007; Zarski et al. 2012; Ljubobratović et al. 2017; Roche et al. 2018).

1.3.2. Control of the reproduction cycle

Photothermic manipulation is widely used to influence gamete maturation and spawning in pikeperch. Photoperiod changes transduced by a photo-neuroendocrine system (retina, suprachiasmatic nucleus and pineal gland) which releases the hormone melatonin exclusively at night (Migaud et al. 2010). The duration of the melatonin release changes according to night length and mediates the transduction of photoperiodic information to the hypothalamus – pituitary-gonad (HPG) axis (Falcón et al. 2010). The hypothalamus acts as an interface between the nervous system and the endocrine system, including internal (e.g. nutritional conditions) as well as external (photoperiod and temperature) triggers (Zohar et al. 2010).

In pikeperch, control of reproduction cycle performed using alteration of photoperiod and temperature (Fontaine et al., 2015) to either (1) accelerate or extend the natural spawning period (advanced or postponed spawning) or (2) to induce an out-of-season spawning. In the first case, broodstock are maintained under natural outdoor conditions (i.e. natural induction of the reproduction cycle), followed transporting to artificial environment for final gamete maturation. In the second case, broodstock are exposed to artificial conditions during whole reproduction cycle with simulation of photo-thermal conditions (Rónyai 2007; Hermelink et al. 2013, 2017; Ljubobratović et al. 2017).

Various hormonal agents are applied aquaculture to synchronize ovulation in pikeperch. Mainly gonadotropins are used as the injections of either carp pituitary extract (CPE), human chorionic gonadotropin (HCG) or gonadotropin releasing hormone agonist (GnRHa) exclusively or in combination with one or two doses (Steffens et al. 1996; Wang et al. 2006; Zakęś 2007;

Zarski et al. 2013). Recently effect of photothermic regime (Hermelink et al. 2013), hand stress (Falahatkar and Poursaeid 2014), and hormonal stimulation (Rónyai 2007; Křišťan et al. 2013; Zarski et al. 2013; Ljubobratović et al. 2017; Roche et al. 2018) on reproduction performance of pikeperch has been studied.

1.3.3. Natural spawning in ponds

The oldest method of pikeperch reproduction is pond spawning (Rónyai 2007; Zakęś and Demska-Zakęś 2009). Pikeperch spawners caught during the pre-spawning season or at spawning grounds and placed to the pond. Depending on latitude, the natural spawning period ranges from April to June (Lehtonen et al. 1996; Lappalainen et al. 2003; Saulamo and Lappalainen 2007; Güralp et al. 2016). Spawning takes place at water temperatures of 12–15°C, usually during the first half of May, depending on the average season temperature. Fingerling pikeperch are harvested in autumn. This method of pikeperch spawning is relatively simple and often doesn't require any special preparation (Steffens et al. 1996), although reproduction performance are very low and final production results are hardly predictable (Steffens et al. 1996; Zakęś and Demska-Zakęś 2009).

1.3.4. Semi-controlled spawning, "nest spawning" or "tank spawning"

Semi-controlled reproduction requires preparation of spawning nests, made of either natural (pine branches, tiny willow roots, and sedge roots) or synthetic materials which are more preferred due to absence of organic matter resulting in better incubation efficiency (Lappalainen et al. 2003; Lehtonen et al. 2006; Zakęś and Demska-Zakęś 2009). A set of spawners (1 female 1–2 males) is placed in a tank with artificial spawning substrate (The nest; Rónyai, 2007; Schlumberger & Proteau, 1996; Steffens et al., 1996; Zakęś & Demska-Zakęś, 2009). A water temperature used for spawning is 14-16 oC (Zakęś and Demska-Zakęś 2009). For spawning in cage spawners are usually stocked into at a density of 2–5 females and 4–10 males and eggs are layed usually after two to three days. Hormonal stimulation is used rather rarely and usually includes gonadotrophic hormones (GtH).

After reproduction, spawners and moved back to the reservoirs (Zakęś 2007). This method of propagation is used in many European countries, including Hungary, Czech Republic and Germany (Steffens et al. 1996; Rónyai 2007; Blecha et al. 2016).

1.3.5. Artificial spawning (stripping)

Artificial reproduction of pikeperch, like it is for most of commercially cultured fish species, requires hormonal induction for gametes ovulation and spawning synchronization (Rónyai 2007; Křišťan et al. 2013; Zarski et al. 2013; Ljubobratović et al. 2017). A positive effect of hormonal treatment on milt quality of pikeperch males has been reported (Blecha et al. 2016).

Pikeperch eggs are stripped and fertilized with milt collected with syringes or catheter (Blecha et al. 2016; Sarosiek et al. 2016; Křišťan et al. 2018). To fertilize 100 g of eggs it is recommended to use 1.0–2.0 ml^3 semen (Křišťan et al. 2013; Blecha et al. 2016), although spermatozoa density may vary so the milt is usually taken from 2 to 3 males (Křišťan et al. 2013).

Adhesiveness can be removed from the eggs by bathing it in a talc-sodium chloride solution (100 g salt+25 g talc+10 L water) for 45 to 60 min (Schlumberger and Proteau 1996; Zarski et al. 2015). Steffens (1996) propose to use fertilization solution of 0.3% NaCl to prevent rapid egg sticking, and after neutralize adhesiveness by washing eggs in alkaline protease

solution (alcalase; 0.5 %). Also possible to use with milk with talc-sodium (Křišťan et al. 2016). Despite high efficiency of alcalase in removing of adhesiveness its application may significantly decrease larval performance (Ljubobratović et al. 2018).

The eggs are incubated in standard Zug jars at a recommended water temperature of 16.0 to 17.0 °C (Steffens et al. 1996). Jars of a volume of 7 L can accommodate from 0.5 to 5.0 L of eggs. The recommended water flow rate is 1.2 L min^{-1} at the beginning of egg incubation and 4.0-5.0 L min^{-1} in later periods (Steffens et al. 1996; Zakęś and Demska-Zakęś 2009; Ljubobratović et al. 2018). Hatching can start after 4–5 days, however, depending on the temperature and agent used for removing of adhesiveness it could be prolonged to 7 days (Ljubobratović et al., 2018). To induce hatching, it is possible to stop the inflow of the incubator, thereby reducing oxygen supply (Ljubobratović et al. 2018).

1.4. Broodstock management of pond cultured pikeperch

Providing of appropriate conditions in artificial, enclosed system to ensure high gamete quality and effective fertilization is a primary aim of the broodstock management (Migaud et al. 2013). Nutritional and environmental protocols for broodstock reared in RAS is still developing (Wang et al. 2009; Hermelink et al. 2017; Ljubobratović et al. 2017), therefore hatcheries production rely on the breeders harvested from nature environments of ponds lakes and rivers (Ljubobratović et al. 2017; Khendek et al. 2018; Křišťan et al. 2018). In contrast to the RAS cultured breeders, broodstock management of pond-cultured fish may only concern the wintering (if fish is harvested in autumn), spawning and post-spawning periods. The main challenge here is to provide "normal" (e.g. natural) conditions for final oocytes maturation, ovulation and spawning. The exposure to artificial conditions may lead to the fail in reproduction, poor egg quality and/or significant decrease of health status, especially for wild and pond-cultured fish (Rónyai 2007; Sarameh et al. 2012; Ljubobratović et al. 2017).

Sensitivity to stress is one of the most undesirable moments of artificial reproduction in pikeperch broodstock (Sarameh et al. 2012, 2013; Hermelink et al. 2013). Broodstock death are often noted during the first few weeks after spawning (Rónyai 2007). Although, some studies have described a low spawners mortality rate (Zakęś and Demska-Zakęś 2009) and was mainly depended on the broodstock origin. The wild and pond cultured fish are more sensitive to handling stress during controlled reproduction compare to the domesticated and RAS cultured broodstock. Broodstock reared from larvae in RAS, had significantly lower post spawning mortality (10 %; Zakęś 2007). The same females held in RAS can reproduce for the subsequent three or four seasons without any negative impact on the quality of the eggs obtained (Zakęś and Demska-Zakęś 2009; Schaefer et al. 2018). Rónyai (2007) reported death of wild female's after stripping within five days after spawning. However, mortality among fish after nest spawning (eggs deposited on substrate) was at 60%. The handling stress and exposure to the artificial environment are considered to be reasons high post spawning losses. Thereby, domestication is recognized as a very important tool of aquaculture, since the selection towards to the less sensitive fish could make reproduction effectiveness more predictable (Sarameh et al. 2012).

Despite mortality, broodstock caught from the natural conditions are the source of high-quality gametes, since the fish grew up in conditions of natural photothermic regime and natural food resources (Ljubobratović et al. 2017; Křišťan et al. 2018). Therefore, establishing of effective measures preventing or reducing mortality can significantly contribute to broodstock management of wild and pond-cultured pikeperch.

Immune system and haematological parameters of blood in pikeperch affected by photoperiod and handling stress during reproduction in captivity (Sarameh et al. 2012). Classical stress hormones in pikeperch blood, catecholamines and corticosteroids (mainly cortisol) are involved in the secondary or tertiary responses in energy mobilization and metabolism, mineral balance and physiological functions (Falahatkar and Poursaeid 2014; Baekelandt et al. 2019). The physiological aspects of stress appear to be important in terms of responses to artificial environment and perhaps the ability of the fish to resist exhaustion. Male pikeperch respond to photoperiod with changes of erythrocyte number, while females exhibited changes of immunity parameters (Milla et al. 2009; Sarameh et al. 2012). The investigation of stress response could be beneficial for understanding of physiological reactions of the fish to reproduction in captivity.

1.5. Aims and objectives

Investigation of reproduction ecology have a strategic importance for pikeperch aquaculture and can be applied for solving practical issues arising during commercial culture. Their implementation in broodstock management may help to develop appropriate conditions for pre-spawning and spawning period, gamete management and incubation. Such conditions, are currently important limiting factors for successful large-scale pikeperch commercial production. Thereby, overall goal of the thesis was to determine ecological and physiological aspects of pikeperch reproduction biology that could be used to improve reproduction technics and culture of pikeperch.

Main objectives of the thesis were:

1) Investigate spawning substrate selection of pikeperch.
2) Evaluate physiological changes in pikeperch broodstock during spawning in controlled conditions.
3) Compare and evaluate the effectiveness of different antifungal treatments on the mortality of pikeperch after spawning.
4) Investigate the effect of water temperature on feeding activity of pikeperch during wintering.

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CHAPTER 2

THE SUBSTRATE SELECTION AND SPAWNING BEHAVIOUR OF PIKEPERCH *SANDER LUCIOPERCA* L. BROODSTOCK UNDER POND CONDITIONS

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The substrate selection and spawning behaviour of pikeperch *Sander lucioperca* L. broodstock under pond conditions

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Abstract

Spawning substrate is a critical factor in reproduction of commercially valuable percids. Substrate preferences of pikeperch *Sander lucioperca* were investigated using three types of artificial spawning substrates: long fibred brush, artificial turf and smooth plastic. There was a significantly higher preference for the brush nest, with thick rigid structures, than for artificial turf, while plastic nests remained unoccupied. The majority of pikeperch pairs (94.4%) spawned within 14 days of the beginning of the experiment. No difference was observed between substrates in time from stocking of fish to nest occupation and spawning. After egg laying, nests were moved to controlled conditions of a recirculation aquaculture system for incubation. Hatching rate and larva production did not differ significantly among tested substrates. Either used substrate was suitable for spawning and incubation in a recirculation aquaculture system. Obtained results can be used for semi-artificial reproduction as well as for the support and control of wild pikeperch stocks in natural habitats.

KEYWORDS
artificial nest, broodstock, egg incubation, reproduction, *Sander lucioperca*

1 | INTRODUCTION

The pikeperch *Sander lucioperca* is a promising fish species for aquaculture production because of its flesh quality and high market value (Blecha, Kristan, Samarin, Rodina, & Policar, 2015; Samarin, Policar, & Lahnsteiner, 2015). Over the past 10 years, pikeperch production in Europe has increased (FAO, 2018; Steenfeldt et al., 2015). However, more than 95% of the market supply has been provided by capture (FAO, 2018; Policar & Adamek, 2013; Steenfeldt et al., 2015), leading to decline of wild populations in Central, East and Northern Europe (Policar et al., 2016). Commercial fisheries relying on natural sources face income instability driven by biological, management and economic factors (Anderson et al., 2017). Pikeperch aquaculture production is variable and insufficient to fulfil market requirements (FAO, 2016). To decrease population decline and expand artificial production, it is necessary to increase reproduction.

Pikeperch is a common inhabitant of fresh and brackish waters in rivers and lakes of the Caspian and Black sea basins (Balon, Momot, & Reiger, 1977; Lappalainen, Dörner, & Wysujack, 2003). It is a nest building phytophilic or litophilic fish, choosing roots, vegetation, sand, gravel or turf as egg-laying substrate (Balon et al., 1977; Feiner & Höök, 2015; Lappalainen et al., 2003). Spawning occurs at water temperatures ranging from 8°C to 16°C and at 1–3 m depth (Lappalainen et al., 2003; Lehtonen, Lappalainen, Kervinen, & Fontell, 2006). Pikeperch form pairs with the males selecting and clearing mud from the spawning site (Feiner & Höök, 2015; Lappalainen et al., 2003) and for guarding the nest until hatching and swim-up of larvae.

Although prey migration and substrate availability are among the most important drivers of pikeperch populations (Koed, Mejlhede, Balleby, & Aarestrup, 2000), studies of artificial spawning substrates

are rare (Čech et al., 2012; Lehtonen, Hansson, & Winkler, 1996; Nash, Hendry, & Cragg-Hine, 1999). Information about substrate selectivity and the minimum quantity of suitable spawning grounds needed to sustain natural pikeperch populations is lacking. Under natural conditions, the absence of shallow water with macrophytes as suitable spawning grounds is a crucial factor limiting reproduction of many fish species, including commercially valuable percids (Čech et al., 2012; Crane & Farrell, 2013; Lehtonen et al., 2006; Nash et al., 1999). The use of artificial spawning substrates can increase pikeperch reproduction, benefitting commercial fisheries as well as decreasing pressure on wild populations.

Artificial spawning substrates are widely used for supporting reproduction of percids (Čech et al., 2012; Crane & Farrell, 2013; Lehtonen et al., 2006). Investigation of spawning substrate preference could help to support natural pikeperch populations through improvement of spawning habitats. The primary objective of this study was to determine spawning substrate preferences of pikeperch broodstock and to clarify some aspects of natural spawning behaviour such as time of day and effect of temperature. The secondary objective was to assess the suitability of the preferred substrates for egg incubation, hatching and subsequent larvae production in RAS.

2 | MATERIALS AND METHODS

2.1 | Fish groups and spawning area

Eighteen adult pikeperch females (W = 2,284 ± 364 g, TL = 604 ± 26 mm) and 18 males (W = 1,216±363 g, TL = 502 ± 45 mm) were used in this study. All broodstock fish were captured from the culture pond of the fish farm Rybarstvi Nove Hrady Ltd. and transferred to an earthen pond of the South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses in Vodnany, Czech Republic for overwintering. At the end of April 2017, six mature pikeperch males releasing milt and six females with oocytes in first and second stages (Zarski et al., 2012) were randomly placed into each of three similar sized earthen ponds 10 m x 5 m x 1 m. To observe a natural pattern of spawning kinetics, spawning was not hormone-induced. Water temperature at one metre depth was measured at hourly intervals with an auto-recording thermometer (Minikin Tie, Environmental Measuring Systems — EMS Brno Ltd. Czech Republic) throughout the trial. The average daily water temperature of the three ponds was calculated to determine whether water temperature affects substrate selection and spawning (Table 2).

2.2 | Artificial spawning substrates

Three types of substrate were used to make circular (Ø = 890 mm) spawning nests: soft brush (bottle brushes) with fibre length 100 mm, artificial turf of 35 mm length and a smooth plastic sheet (Figure 1). One week before stocking with fish, six nests of each type were placed on the bottom of each pond to provide the potential for each pair to choose each substrate type. Nests were placed on the pond bottom in a staggered arrangement separated by 1 m.

2.3 | Spawning

The parameters observed were as follows: time from fish stocking to male occupation of a nest; time from stocking to spawning; and time

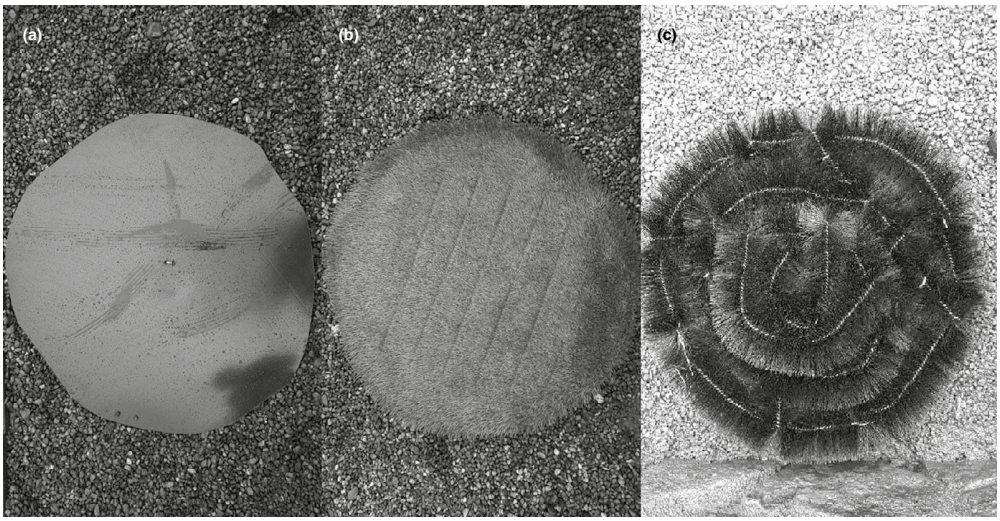

FIGURE 1 Photograph of materials used for investigation of spawning substrate preference of pikeperch *Sander lucioperca*. A—smooth plastic; B—artificial turf; C—brush

from male occupation of nest to spawning. Nests were lifted with cord to the surface for inspection daily at 07.00, 11.00, 15.00 and 19.00 hr. Spawning success was calculated as percentage of the eighteen pairs.

2.4 | Incubation, hatching rate and production of larvae per pair

Twenty-four hours after detection of the spawning, nests with attached eggs were moved to a 360 L cylindrical tank in a room equipped with a RAS with water temperature 15°C for incubation under controlled conditions. The hatching rate (%) was investigated from 100 randomly selected eggs which were separated from each nest immediately after moving to the RAS and divided equally between two 250-ml plastic jars. The jars were installed in a RAS and incubated under conditions similar to the nests. When the first hatched larvae were observed, water flow was stopped, and only aeration was provided via an air stone in each tank. Ninety-six hours after initiation of hatching, when hatching was complete, nests were removed from the tanks, and larvae were concentrated in a 10 L volume in a 20-L tank for counting (Blecha, Samarin, Kristan, & Policar, 2016). A number of larvae in plastic jars were counted manually for determination of hatching rate (%; Policar et al., 2011).

2.5 | Statistical analysis

To confirm the normal distribution of data, Shapiro–Wilk's test was performed. When normal distribution was confirmed, differences in time intervals from stocking to occupation and spawning, hatching rate and larva production among substrates were estimated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The Kruskal–Wallis nonparametric ANOVA was used for evaluation of substrate preference. Unoccupied nest types were excluded from statistical analysis. A full factorial design in general linear models was used to investigate whether water temperature influenced substrate preference. The most suitable model was determined by gradual deletion of the less significant parameters or interactions from the general model. Spawning kinetics was described by a simple linear regression using data sets transformed to a cumulative percentage of spawning pairs relative to the day of the experiment. Changes in water temperature throughout the experiment were analysed with simple linear regression. Analyses were conducted in Statistica 13 (StatSoft). For all tests, the level of significance was set at $p < 0.05$ with results presented as mean ± *SD*.

3 | RESULTS

3.1 | Spawning success and substrate preference

Fourteen days after beginning of the experiment, 17 of 18 pikeperch pairs had spawned, and spawning success accounted 94.4%. A significantly higher preference ($p < 0.05$) was found for brush (61.1% ± 9.6%) than for artificial turf (33.3% ± 0%) (Table 1). Plain plastic sheet materials were not occupied.

3.2 | Spawning kinetics

The majority of broodstock (94.4%) spawned within 14 days of stocking into the experimental ponds. The daily increase in the percentage of spawners was significantly higher in brush at 5.6% ± 0.4% (*M*; $F_{1,12} = 140.85$; $p < 0.05$) compared to turf at 3.5% ± 0.2% ($F_{1,12} = 33.92$; $p < 0.05$) (Figure 2). Throughout the experiment, 65% of spawning events occurred between 07.00 and 11.00 hr and 18% spawned between 19.00 and 07.00 hr. Spawning during the day was 12% and 6% for the periods from 11.00 to 15.00 hr and 15.00 to 19.00 hr respectively. The peak of spawning occurred after day six. By day ten, spawning activity was reduced, and only three pairs spawned in the final days of the experiment (Figure 2).

The time from broodstock stocking to male occupation was 96.5 ± 45.5 hr in brush and 98.5 ± 17.9 hr in turf. Time between the first male occupation of a nest and spawning was 79.5 ± 77.1 hr in brush and 48.7 ± 52.2 hr in turf nests. Time from stocking to spawning was 167.4 ± 72.8 hr in the brush nests and 147.2 ± 46.3 hr in turf (Table 1). There were no significant differences in all time parameters between nesting substrates.

3.3 | Water temperature and substrate preference

The water temperature differed significantly over the course of the experiment ranged from 8.3°C to 12.2°C ($p < 0.05$; Table 2).

TABLE 1 Spawning, hatching rate and larva production from pikeperch *Sander lucioperca* nests. Mean ± *SD*

Nest material	Pairs	Use of nest (%)	Hatching rate (%)[a]	Larvae, (thousands)[b]	Time from occupation — spawning, hr[c]	Time from occupation — spawning, hr[c]	Time from stocking — spawning, hr[c]
Brush	11	61.1 ± 9.6	71.8 ± 23.1	200.3 ± 92.6	96.5 ± 45.5	79.5 ± 77.1	167.4 ± 72.8
Artificial turf	6	33.3 ± 0	72.8 ± 21.4	180.2 + 104.7	98.5 ± 17.9	48.7 + 52.2	147.2 ± 46.3
Smooth plastic	0	0.0	–	–	–	–	–
No spawning	1	5.6	–	–	–	–	–
Total	18	100.0					

[a]Hatching rate ($F_{1,\ 15} = 0.0078$; $p > 0.05$). [b]Larvae, (thousands) ($F_{1,\ 15} = 0.16671$; $p > 0.05$). [c]Time periods from stocking-occupation-spawning ($F_{3,\ 13} = 0.26$; $p > 0.05$)

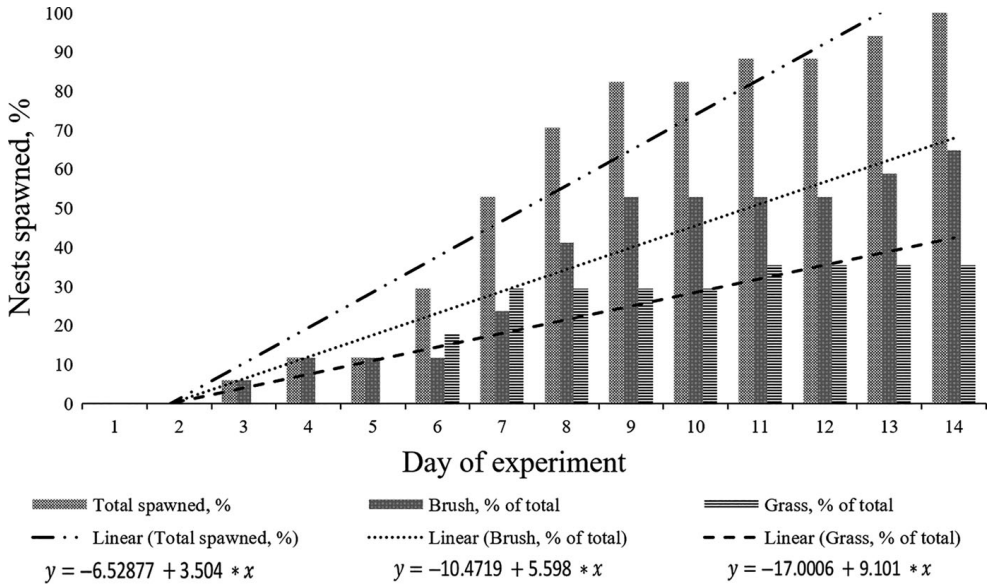

FIGURE 2 Spawning kinetics and substrate preference during natural reproduction period in April 2017 are shown as the cumulative percentage of spawned pikeperch *Sander lucioperca* pairs relative to day of experiment

The general linear model showed higher water temperature to be significantly associated with spawning occurrence ($F_{1,24}$ = 32.27; $p < 0.05$) but not substrate preference. The preference for brush substrate was consistent throughout the experiment and was not influenced by water temperature or nest availability ($p > 0.05$).

3.4 | Incubation success

There was no significant difference in hatching rate between samples taken from tested substrates: brush = 71.8% ± 23.1%; turf = 72.8% ± 21.4% or larva production, brush = 200 300 ± 92 600 psc; turf = 180 200 ± 104 700 psc (Table 1).

4 | DISCUSSION

Pikeperch is a key predator of many aquatic ecosystems. Its predatory effect has been used for several ecological purposes mainly as biomanipulation for elimination of unwanted fish species from the natural water bodies (Adamek & Opacak, 2005). Decline in natural pikeperch populations within recent decades caused by overfishing and ecosystem degradation has led to ecosystem overcrowding by small cyprinids (roach—*Rutilus rutilus*, rudd—*Scardinius erythrophthalmus* or Topmouth Gadeon—*Pseudorasbora parva*; Adamek & Opacak, 2005). It is necessary to indicate and supplement spawning sites in natural water bodies to increase the natural production of pikeperch populations (Lehtonen et al., 2006).

In the wild, pikeperch prefers dense structures for spawning and egg laying such as plant roots and branches or sometimes gravel or sand (Lappalainen et al., 2003). Descriptions of natural spawning sites are generally vague references to "roots, branches, and plants" with size about 500 mm in a diameter and 50–100 mm thick (Lappalainen et al., 2003; Zakes & Demska-Zakes, 2009). We found a preference for the thick rigid structure of the brush nest over soft artificial turf fibres, while smooth plastic remained unoccupied. This is the first study of pikeperch concerning selectivity to type and structure of artificial substrates.

Spawning nests are also widely used for controlled pikeperch reproduction (Demska-Zakes & Zakes, 2002; Lappalainen et al., 2003). They are made of both natural and synthetic materials (Luczynski et al., 2007) to imitate natural spawning substrates. As the structure matters more the material of the nest (Čech et al., 2012; Crane & Farrell, 2013; Lehtonen et al., 2006), it is a good reason for synthetic materials to be used to prevent water contamination with organic matter during nest incubation in RAS. Artificial nests are usually square or round and measure approximately 500 × 500 mm 0.25 m^2 (Lappalainen et al., 2003; Luczynski et al., 2007; Steenfeldt et al., 2015; Zakes & Demska-Zakes, 2009). Salminen and Ruuhijärvi (1991) suggested that 0.42 m^2 (650 × 650 mm) nests are sufficiently large for pikeperch females to deposit eggs, but

TABLE 2 Water temperature dynamic during 14 days of the experiment. Mean ± *SD*

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Temperature, °C	9.4 ± 1.23	9.6 ± 0.6	10.4 ± 0.42	8.3 ± 0.95	8.6 ± 0.81	10.4 ± 1.11	11.9 ± 0.85	11.3 ± 0.5	11.7 ± 1.15	12.2 ± 0.71	12 ± 0.63	10.9 ± 0.4	10.7 ± 1.56	12.2 ± 1.68

too small for use by fish above 2,500 g. Steffens, Geldhauser, Gerstner, and Hilge (1996) used nests of 1800 × 700 mm (surface area 1.26 m^2) for spawning in cages, with stocking density of 4–10 males and 2–5 females per cage. We used circular nests of 0.79 m^2 area (Ø = 890 mm), which corresponded to the size of the tanks used for egg incubation. This size allowed to obtain good egg distribution from females 2000–2,200 g of weight; however, to prevent egg deposition out of the artificial nest, it might be suggested to use nest with diameter >1,000 mm for the females heavier than 2,500 g. Nests for larger females should be sized accordingly, to protect eggs from conglutination and resulting fungal infection during incubation (Luczynski et al., 2007).

Fungal infections present a challenge to the use of artificial nests for pikeperch egg incubation (Zarski, Horvath, Held, & Kucharczyk, 2015), which are primarily the result of clustering and conglutination of the egg mass (Kucharczyk et al., 2007; Luczynski et al., 2007). Natural substrates including juniper and conifer branches, rice turf and wood wool have been used (Kucharczyk et al., 2007; Skrzypczak, Kucharczyk, Mamcarz, Kujawa, & Furgala-Selezniow, 1998); however, degradation of natural materials may produce a suitable medium for fungal infections (Zarski et al., 2015) as well as increase organic matter in water and reduce incubation success. We found synthetic materials to support water conditions without organic contamination along with appropriate egg distribution to provide high hatching rates and larva production for brush and artificial turf nests respectively.

Eggs for evaluation of the hatching rate were separated from the spawning substrate one day after determination of the spawning occurrence; thus, there was no effect of particular spawning substrate on the incubation and hatching. The percentage of successfully hatched larvae was in range of 48%–94%. The results are in line with previous studies reported about hatching rate from pond cultured pikeperch broodstock in range of 35%–95% (Blecha, Flajshans, et al., 2016; Ljubobratovic et al., 2017).

Pikeperch mating and egg laying are described as 20–25 min of courtship characterized by swimming around the nest and spawning (10–15 min: Lappalainen et al., 2003). There is no research concerning at what point of the reproductive cycle the male select and clean the spawning site. Present study found the nonsignificant trend to longer time from the male occupation to spawning in brush nests indicated that these nests may be more attractive for broodstock compared to the artificial turf nest with shorter preparation and invitation period. Thus, the preparation time for preferred nests was longer, although not significantly, compared to the less preferred nest. This finding did not affect other parameters of pikeperch reproduction; however, it may be interesting from an ecological point of view. Longer preparation could have a reflection in fertilization rate as the spawning substrates are suspected to stimulate the papilla of the male during nest cleaning.

Although hormone stimulation of pikeperch broodstock is often recommended in aquaculture to synchronize spawning (Kristan, Alavi, Stejskal, & Policar, 2013; Samarin, Blecha, Bytyutskyy, & Policar, 2015; Zarski et al., 2015) in this study, spawning was not hormone-

induced which allowed us to observe a natural pattern of spawning kinetics. We found that pikeperch mostly preferred morning hours: 18% of pairs spawned from 19.00 to 07.00 hr, while 65% spawned between 07.00 and 11.00 hr in the morning. Our results corresponded to Schlumberger and Proteau (1996) reporting that spawning usually takes place during night and early morning hours.

One of the most important factors influencing pikeperch reproduction biology is temperature (Hermelink et al., 2011; Hermelink, Wuertz, Rennert, Kloas, & Schulz, 2013; Hokanson, 1977). Pikeperch has a highly plastic temperature range for spawning, with reported preferred water temperature from 8°C to 18°C (Hermelink et al., 2011; Hokanson, 1977; Lappalainen et al., 2003). During our observation, pikeperch successfully spawned at 8.3°C to 12.2°C (Table 2). Water temperature commonly used for spawning induction during controlled, semi-controlled reproduction and egg incubation is 14–15°C (Blecha, Kristan, & Policar, 2016; Hermelink at al., 2011; Kristan et al., 2013; Ljubobratovic et al., 2017). Statistical analysis confirmed an interaction of temperature rise with spawning occurrence. Our results are in line with Hokanson (1977) who emphasized a significant role of water temperature in spawning of pikeperch.

Research concerning pikeperch reproduction biology, kinetics and behaviour is needed. There is a lack of information with respect to natural spawning in pond and lake conditions, which may be used for supporting wild stocks, as well as for increasing efficiency of semi-artificial reproduction of pikeperch. Another important aspect of pikeperch spawning behaviour with respect to the spawning sites is homing. Although return of adult fish to particular spawning areas has been confirmed by tagging (Lappalainen et al., 2003), there is no research investigated whether the fish were hatched in those areas. In the example of the relative species Walleye, natal site fidelity has been indicated by genetic differences among spawning groups (Stepien, Banda, Murphy, & Haponski, 2012).

Obtained results could be used for increasing of pikeperch spawning opportunities in natural conditions by adding artificial substrate to shallow areas of open waters, as has been performed for related percid fishes (Crane & Farrell, 2013; Nash et al., 1999). Investigation of substrate selectivity and the necessary quantity of suitable spawning grounds may be used for restocking purpose, support and sustaining of natural pikeperch populations. Artificial spawning substrate can be used in semi-controlled reproduction and production of larvae for restocking of natural waters, as well as for pond and RAS culture.

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CHAPTER 3

BEHAVIOR AND PHYSIOLOGICAL STATUS OF POND-CULTURED PIKEPERCH (*SANDER LUCIOPERCA*) BROODSTOCK EFFECTED BY SEXUAL INTERACTIONS THROUGHOUT SEMI-ARTIFICIAL REPRODUCTION

Malinovskyi, O., Kolářová, J., Blecha, M., Stará, A., Velíšek, J., Křišťan, J., Policar, T., 2019. Behavior and physiological status of pond-cultured pikeperch (*Sander lucioperca*) broodstock effected by sexual interactions throughout semi-artificial reproduction. Aquaculture International. DOI: 10.1007/s10499-019-00401-6

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EUROPEAN PERCID FISH CULTURE

Behavior and physiological status of pond-cultured pikeperch (*Sander lucioperca*) broodstock effected by sexual interactions throughout semi-artificial reproduction

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Abstract
This study investigated physiological and behavioral responses of pikeperch broodstock related to sexual interactions under controlled conditions throughout semi-artificial reproduction. Blood samples taken from pond-cultured pikeperch broodstock were analyzed to assess the stress response at different stages of semi-artificial reproduction protocol. Sampling was performed as follows: before hormonal induction (control), 24 h (SP24) and 48 h (SP48) after spawning when males and females were separated, and 24 h (NS24) and 48 h (NS48) after spawning when males and females were kept together. The separation immediately after spawning had a significant effect on physiological state of broodstock. Separation affected indices of erythrocyte count, hematocrit (packed cell volume, PCV), glucose (GLU), and mean corpuscular volume (MCV) in females and levels of lactate and leukocytes in males. Monitoring of pikeperch behavior in groups NS24 and NS48 revealed the strong aggressive paternal behavior of male to females. Attacks mainly targeted the caudal fin and resulted in female mortality in group NS48.

Keywords Aquaculture · Blood analyzing · Ecology · Management · Mortality · Spawning

Introduction

Over the last few decades, commercial interest in pikeperch culture prioritized research developing reproduction techniques (Blecha et al. 2016; Policar et al. 2016; Samarin et al.

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2015). Despite current progress in domestication, wild-caught and pond-cultured pikeperch are still a source of broodstock providing high-quality gametes (Fontaine et al. 2009, 2015; Křišťan et al. 2012, 2018; Ljubobratović et al. 2017; Khendek et al. 2018; Kestemont and Henrotte, 2015). However, the biggest disadvantage of wild and pond-cultured broodstock is their higher post-spawning mortality in comparison to broodstock reared in recirculation aquatic systems (RASs) (Łuczyński et al. 2007; Rónyai 2007; Zakęś and Demska-Zakęś 2009; Zakęś et al. 2013).

Semi-artificial or so-called "nest" spawning is an effective and convenient reproduction method for pond-cultured and wild broodstock. Unlike artificial reproduction (stripping), where post-spawning losses may reach up to 100% in several days after spawning (Gomułka et al. 2007; Łuczyński et al. 2007; Rónyai 2007; Zakęś and Demska-Zakęś 2009). Semi-artificial reproduction in RASs and ponds (Malinovskyi et al. 2018) has significantly less handling requirements, and broodstock survival after recovery treatment can be about 92–100% depending on the sex (Policar et al. 2019).

Stress response is a generically costly process resulting in poor physiological status of broodstock (Schreck et al. 2001; Schreck 2010; Sarameh et al. 2013). Post-spawning losses of pikeperch are mainly caused by exposure to artificial environment and handling procedures resulting in a decrease of immunity level and subsequent outbreak of either protozoan, bacterial, or fungal infections (Gomułka et al. 2007; Muller-Belecke and Zienert 2008; Zakęś and Demska-Zakęś 2009). Antifungal and antibacterial treatments could increase survival (Policar et al. 2019), even though their application aimed to resolve secondary consequences, like diseases, rather than improvement of the poor health status.

Physiological changes and stress responses of pikeperch broodstock on artificial photoperiod, hormonal treatment, and handling were studied during nest or artificial spawning by Sarameh et al. (2012, 2013) and Falahatkar and Poursaeid (2014); however, broodstock physiology and stress response were not documented in relation to broodstock behavioral interaction between males and females. General spawning behavior before and during nest spawning in pikeperch was observed by Drasovean and Blidariu (2013), but neither of the male's aggression during spawning nor problematic interactions between sexes after spawning were mentioned there.

The aims of this study were (1) to investigate the health and physiological status of pikeperch broodstock during semi-controlled reproduction, (2) to determine the most stressful steps related to broodstock sexual behavior, and finally, (3) to optimize broodstock management in pond-cultured pikeperch during semi-artificial reproduction.

Materials and methods

Fish and experimental conditions

In total, 15 males (total length (TL) = 542 mm ± 37 mm and body weight (BW) = 1487 g ± 434 g) and 15 females (TL = 532 mm ± 40 mm and BW = 1353 g ± 309 g) of matured pikeperch were used in this study. All broodstock fish were captured from the production pond of the fish farm Rybářství Nové Hrady, Ltd., during the autumn harvesting season, before being transferred to an earthen pond at the Faculty of Fisheries and Protection of Waters, University of South Bohemia in Vodňany, Czech Republic, for overwintering. In early April, males and females were randomly selected for the study and stocked into a RAS. Fish were

evenly distributed among six tanks with a volume of 350 l—three for males and three for females. After 2 weeks of adaptation, pikeperch broodstock were divided into pairs for sampling throughout reproduction (described in Table 1). Each pair of pikeperch was stocked into separate 350-l tanks connected to a RAS. Throughout the experiment, fish were exposed with artificial luminescent light. The intensity was set on 50–100 lx on water surface with the photoperiod of 13 h light and 11 h dark (13L:11D) in accordance with the latitude of the Czech Republic (at the end of April). Water temperature was constantly set at 15 °C ± 0.3 °C (Blecha et al. 2015), and oxygen saturation was maintained at a level of 107% ± 2.7%. The nests, made from artificial grass, were placed on the bottom of each tank as a spawning substrate (Malinovskyi et al. 2018). For the induction and synchronization of spawning, both sexes were intramuscularly injected with the human chorionic gonadotropin (hCG) (Chorulon; Intervet, Netherlands) at a concentration of 500 IU per kg (Křišťan et al. 2013).

All the manipulations with fish during the experiment were done under anesthesia (clove oil, 0.03 ml l^{-1}; according to Křišťan et al. 2014) in accordance with the directive 2010/63/EU on the protection of animals used for scientific purposes. The injection of hormonal agents and samplings of the blood were performed with veterinary guidance in accordance with good veterinary practice. The fish in this experiment have not been used more than once in procedures involving pain, distress, or suffering.

Blood sampling

Three pairs of fish were sampled on different stages of semi-artificial reproduction protocol: before hormonal treatment and 24 h and 48 h after spawning (Table 1). After anesthetizing the fish, blood samples were collected from vena caudalis using a heparinized needle and 1-ml volume syringe. Immediately after collection, the blood was transferred to microtubes rinsed with sodium heparin (Heparin Léčiva inj. sol.; Zentiva, Prague, Czech Republic) 40 IU per ml of blood to prevent coagulation.

The blood samples for biochemical analyses were immediately centrifuged at 1500×*g* for 10 min in a microcentrifuge (MPW 55; MPW Instruments, Warszawa, Poland) after sampling, and the blood plasma was transferred on ice and stored in − 80 °C before analyses. The blood samples for hematological analyses were processed immediately after sampling. After the

Table 1 Description of experimental groups and sampling plan of pikeperch (*Sander lucioperca*) broodstock throughout semi-artificial reproduction

Group name	Group no.	*n*	Sex (M/F)	Hormonal treatment applied (yes/no)	Description
Control	1	3	F	No	Fish were sampled at the beginning of the experiment, before hormonal treatment
	2	3	M	No	
NS24	3	3	F	Yes	Fish were sampled 24 h after spawning; males and females were not separated
	4	3	M	Yes	
NS48	5	3	F	Yes	Fish were sampled 48 h after spawning; males and females were not separated
	6	3	M	Yes	
SP24	7	3	F	Yes	Fish were sampled 24 h after spawning; males and females were separated
	8	3	M	Yes	
SP48	9	2	F	Yes	Fish were sampled 48 h after spawning; males and females were separated
	10	3	M	Yes	

NS not separated, *SP* separated, *M* male, *F* female, *h* hours

sampling procedure, fish were transferred into a RAS with a constant water salinity of 5–10 g l^{-1} of NaCl for elimination of secondary fungal infection (Policar et al. 2019). Comparison of hematological and biochemical indices was used for evaluation of physiological changes during semi-artificial reproduction with respect to sexual behavior with the aim of optimizing the separation of females after spawning.

Measurement of hematological and biochemical parameters

The hematological profile was evaluated immediately after blood sampling according to Svobodova et al. (2012) and included an erythrocyte (Er) count, hematocrit (packed cell volume, PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and leukocyte (Leuko) count. Cortisol was assayed in the blood plasma by RIA using a commercial antibody kit (cortisol-3-OCMO antiserum; Immunotech, Prague, Czech Republic). The following biochemical parameters in the blood plasma: calcium (Ca^{2+}), glucose (GLU), and lactate (LACT) were assayed by the methods described by Kolářová and Velíšek (2012) using the blood gas analyzer VetTest 8008 (IDEXX Laboratories, Inc., USA).

Monitoring of pikeperch behavior after spawning and evaluation of fin erosion

A waterproof digital camera (LAMAX Action X8 Electra; elem6 s.r.o., Czech Republic) was used for observing pikeperch behavior after nest spawning. Five-minute recordings were randomly collected during the light phase within the following periods: 0–24 h, 24–48 h, and 48–72 h post spawning in groups NS24 and NS48. The total duration of records accounted 135 min for each 0–24-h and 24–48-h period and 120 min for the period 48–72 h after spawning. After recording, completed video files were analyzed to determine specific patterns of sexual behavior after spawning such as the number of male attacks on female per minute.

The caudal fins of both sexes in all groups were photographed and processed throughout the experiment. Evaluation of fin erosion was done by image analysis with QuickPHOTO MICRO 3.0 software. Fin erosion was shown as a percentage of the fragmented and inflamed area of the total caudal fin surface (according to Policar et al. 2016).

After the experiment, all fish were transferred to a RAS with brackish water in a concentration of 5–10 g l^{-1} for recovery and were fed ad libitum with prey fish (*Pseudorasbora parva*) according Policar et al. (2019). After 2 weeks of the recovery period, pikeperch broodstock were restocked to the local polyculture pond.

Statistical analysis

Data from 15 pikeperch males and 14 females were analyzed due to the mortality of one female at the end of the experiment. Due to the rareness of matured pikeperch during spawning season, it was possible to get samples of only three individuals for each tested group. Although the test strength of some data points was not high, it was enough to find significant differences among groups in the following parameters.

The normal distribution of data was ensured by the Shapiro–Wilk test. Normal distribution of caudal fin erosion data was achieved by square root transformation. After the parametric assumptions were met, differences in hematological, biochemical, and caudal fin erosion values were estimated using two-way analysis of variance (ANOVA) followed by post hoc

Table 2 Comparison of hematological indices in pikeperch (*Sander lucioperca*) sampled on different steps of the semi-artificial reproduction protocol (groups described in Table 1)

Group name	Group no.	Sex (M/F)	Sampled individuals	Hb (g l^{-1})	PCV ($l\,l^{-1}$)	Er ($T\,l^{-1}$)	MCV (fl)	Leuko (g l^{-1})
Control	1	F	3	58.01 ± 4.12 [5, 7]	0.47 ± 0.07 [3–10]	1.80 ± 0.28	259.91 ± 31.04 [4, 6–10]	14.17 ± 1.03 [8, 10]
	2	M	3	58.13 ± 4.44 [5, 7]	0.44 ± 0.02 [3–10]	1.77 ± 0.11	248.07 ± 8.38 [4, 6–9]	6.67 ± 3.47 [5, 9]
NS24	3	F	3	49.89 ± 2.88	0.33 ± 0.02 [1, 2, 7]	1.48 ± 0.04 [4, 6]	222.77 ± 9.37 [7, 8]	11.50 ± 1.87
	4	M	3	57.41 ± 1.61 [5, 7]	0.35 ± 0.04 [1, 2, 7]	1.86 ± 0.18 [3, 5]	187.29 ± 19.61 [1, 2, 7]	11.50 ± 5.72
NS48	5	F	2	44.76 ± 5.37 [1, 2, 4, 10]	0.31 ± 0.06 [1, 2]	1.43 ± 0.16 [4, 6]	217.48 ± 16.69 [7, 8]	19.00 ± 3.50 [2, 8, 10]
	6	M	3	49.89 ± 3.93	0.34 ± 0.02 [1, 2, 7]	1.90 ± 0.15 [3, 5]	181.13 ± 24.4 [1, 2, 7]	14.33 ± 6.25 [8, 10]
SP24	7	F	3	46.19 ± 6.53 [1, 2, 4, 10]	0.23 ± 0.02 [1, 4, 6, 9, 10]	1.75 ± 0.09	133.83 ± 13.56 [1–6, 9–10]	12.33 ± 1.84 [8]
	8	M	3	52.52 ± 5.31	0.28 ± 0.04 [1, 2, 9]	1.78 ± 0.11	156.99 ± 24.82 [1–3, 5, 10]	3.67 ± 1.25 [1, 5–7, 9]
SP48	9	F	3	52.28 ± 4.5	0.36 ± 0.004 [1, 2, 7, 8]	1.80 ± 0.06	198.00 ± 48.16 [1, 2, 7]	18.17 ± 5.33 [2, 8, 10]
	10	M	3	56.82 ± 6.43 [5, 7]	0.34 ± 0.02 [1, 2, 7]	1.64 ± 0.31	210.57 ± 31.33 [1, 7, 8]	5.50 ± 2.45 [1, 5, 6, 9]

Superscript numbers 1–10 indicate the observed significance levels of $p < 0.05$, compared to other tested groups. All values are presented as means ± SD. Probabilities for post hoc tests are as follows: for Hb, MS = 33.906, $df = 19$; for PCV, MS = 0.00183, $df = 19$; for Er, MS = 0.04453, $df = 19$; for MCV, MS = 652.92, $df = 19$; and for Leuko, MS = 21.447, $df = 19$

NS not separated, *SP* separated, *M* male, *F* female, *Hb* hemoglobin, *PCV* packed cell volume (hematocrit), *Er* erythrocyte, *MCV* mean corpuscular volume, *Leuko* leukocytes

Table 3 Comparison of biochemical indices in pikeperch (*Sander lucioperca*) sampled on different steps of the semi-artificial reproduction protocol (groups described in Table 1)

Group name	Group no.	Sex (M/F)	Sampled individuals	Cortisol (ng ml^{-1})	Ca^{2+} (mmol l^{-1})	GLU (mmol l^{-1})	LACT (mmol l^{-1})
Control	1	F	3	228.90 ± 96.65 [2]	3.30 ± 0.26	7.97 ± 4.23 [7]	2.53 ± 0.18 [10]
	2	M	3	68.34 ± 17.47 [1, 7, 9]	2.97 ± 0.42	7.55 ± 3.95	3.00 ± 0.33 [10]
NS24	3	F	3	211.03 ± 125.82	3.01 ± 0.22	5.71 ± 1.90	2.96 ± 0.39 [10]
	4	M	3	194.69 ± 20.82	2.32 ± 0.16 [6]	8.02 ± 2.98 [7]	3.55 ± 0.26 [10]
NS48	5	F	2	133.11 ± 14.47	2.91 ± 0.74	8.84 ± 5.84 [7]	2.50 ± 0.60 [10]
	6	M	3	82.41 ± 66.03 [7]	4.18 ± 2.36 [4, 8, 10]	5.11 ± 1.92	2.32 ± 0.49 [10]
SP24	7	F	3	276.60 ± 86.87 [2, 6, 10]	3.85 ± 0.27	1.94 ± 0.28 [1, 4, 5]	1.54 ± 0.31 [10]
	8	M	3	220.28 ± 81.45	2.25 ± 0.22 [6]	4.62 ± 1.16	3.19 ± 0.37 [10]
SP48	9	F	3	235.41 ± 70.81 [2]	3.87 ± 0.29	3.11 ± 0.79	1.83 ± 0.44 [10]
	10	M	3	89.49 ± 60.34 [7]	2.35 ± 0.15 [6]	4.44 ± 0.13	5.80 ± 2.85 [1–9]

Superscript numbers 1–10 indicate the observed significance levels of $p < 0.05$, compared to other tested groups. All values are presented as means ± SD. Probabilities for post hoc tests are as follows: for cortisol, MS = 8407.3, $df = 19$; for Ca^{2+}, MS = 1.0229, $df = 19$; for GLU, MS = 11.763, $df = 19$; and for LACT, MS = 1.4852, $df = 19$

NS not separated, *SP* separated, *M* male, *F* female, Ca^{2+} calcium, *GLU* glucose, *LACT* lactate

Tukey tests to find the differences among tested groups. All analyses were conducted using the statistical package Statistica 13 (StatSoft, Inc.). For all tests, the level of significance was set at $p < 0.05$. Results were presented as mean ± SD. Statistical differences during reproduction are marked in Tables 2 and 3 according to the numbers of fish groups (described in Table 1).

Results

Hematological profile

Changes of hematological profile in broodstock of each group are summarized in Table 2. Indices of Hb in the blood of females were significantly lower ($p < 0.05$) in groups NS48 (44.76 g l^{-1} ± 5.37 g l^{-1}) and SP24 (46.19 g l^{-1} ± 6.53 g l^{-1}) compared to the control group (58.01 g l^{-1} ± 4.12 g l^{-1}), while there were no significant differences in males among tested groups. Indices of PCV were significantly lower ($p < 0.05$) in all tested groups in comparison to control groups in both males and females.

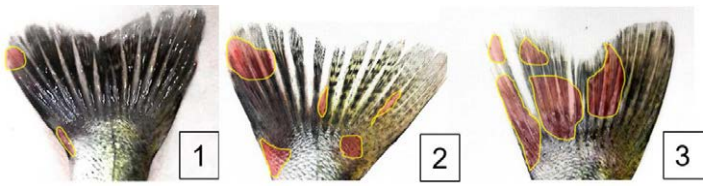
Indices of Er count in blood had significant differences ($p < 0.05$) only between the males and females from groups NS24 and NS48 and accounted the following: in NS24, 1.86 T l^{-1} ± 0.18 T l^{-1} for males and 1.48 T l^{-1} ± 0.04 T l^{-1} for females, and in NS48, 1.90 T l^{-1} ± 0.15 T l^{-1} for males and 1.43 T l^{-1} ± 0.16 T l^{-1} for females.

Values of MCV in females were significantly lower ($p < 0.05$) in groups SP24 (133.83 fentoliters (fl) ± 13.56 fl) and SP48 (198.00 fl ± 48.16 fl) compared to groups NS24 (222.77 fl ± 9.37 fl) and NS48 (217.48 fl ± 16.69 fl) and the control group (259.91 fl ± 31.04 fl). Indices of MCV in males varied with the same trend and were significantly lower in groups NS24 (187.29 fl ± 19.61 fl), NS48 (181.13 fl ± 24.4 fl), and SP24 (156.99 fl ± 24.82 fl) when compared to the control group.

Indices of Leuko count in the blood of females had no significant differences among tested groups. Values of Leuko count in males were significantly higher in group NS48 (14.33 g l^{-1} ± 6.25 g l^{-1}) in comparison with groups SP24 (3.67 g l^{-1} ± 1.25 g l^{-1}) and SP48 (5.50 g l^{-1} ± 2.45 g l^{-1}). There were no significant differences in indices of MCH and MCHC among tested groups.

Biochemical plasma profile

Changes in indices of biochemical plasma profile of broodstock are summarized in Table 3. Females' level of cortisol was significantly higher ($p < 0.05$) in comparison to males in the



1 - Minimal erosion (<3 % of fin surface is damaged).
2 - Minor erosion (3-15 % of surface is damaged).
3 - Moderate erosion (15-25% of fin surface is damaged).

Fig. 1 The examples of different levels of erosion in caudal fin of pikeperch (*Sander lucioperca*) broodstock after semi-artificial reproduction. 1 Minimal erosion (< 3% of fin surface is damaged). 2 Minor erosion (3–15% of surface is damaged). 3 Moderate erosion (15–25% of fin surface is damaged)

Table 4 Level of caudal fin erosion in pikeperch (*Sander lucioperca*) broodstock on different steps of semi-artificial reproduction (groups described in Table 1)

Group name	Sex	
	Male (%)	Female (%)
NS24	2.5 ± 0.4	15.7 ± 3.5*
NS48	1.9 ± 0.6	23.8 ± 2.9*
SP24	0.7 ± 0.4	2.7 ± 0.6*
SP48	2.7 ± 1.1	2.3 ± 0.4

All values are presented as means ± SD

NS not separated, *SP* separated

*$p < 0.05$, significant levels observed

control group alone (68.34 ng ml^{-1} ± 17.47 ng ml^{-1} for males and 228.90 ng ml^{-1} ± 96.65 ng ml^{-1} for females). Although the given high variability in cortisol values, there was no significance in tested groups, although the general baseline of cortisol level was higher in females compared to the males.

Indices of Ca^{2+} in the plasma of females varied without significant differences among tested groups. Values of Ca^{2+} in the plasma of males from group NS48 (4.18 mmol l^{-1} ± 2.36 mmol l^{-1}) were significantly higher ($p < 0.05$) compared to those from groups NS24 (2.32 mmol l^{-1} ± 0.16 mmol l^{-1}), SP24 (2.25 mmol l^{-1} ± 0.22 mmol l^{-1}), and SP48 (2.35 mmol l^{-1} ± 0.15 mmol l^{-1}).

Values of GLU in the plasma of females from group SP24 (1.94 mmol l^{-1} ± 0.28 mmol l^{-1}) were significantly lower ($p < 0.05$) compared to group NS48 (8.84 mmol l^{-1} ± 5.84 mmol l^{-1}) and the control group (7.97 mmol l^{-1} ± 4.23 mmol l^{-1}). Indices of GLU in the plasma of males were not significantly different among tested groups.

LACT plasma indices of females did not differ significantly among tested groups, while blood LACT indices of males were significantly higher ($p < 0.05$) in group SP48 (5.80 mmol l^{-1} ± 2.85 mmol l^{-1}) compared to all tested groups.

Monitoring of pikeperch behavior after spawning and evaluation of fin erosion

After spawning, all males exhibited strong aggressive paternal behavior to females in groups NS24 and NS48. There was no evidence of female aggressive behavior, and all attacks were initiated by males. The attack frequency accounted 1.68 ± 1.46 attacks per min within 0–24 h after spawning, 2.26 ± 1.14 attacks per min within 24–48 h after spawning, and 2.56 ± 2.36 attacks per min within 48–72 h after spawning without significant differences between groups. The caudal fin and tail of females were the most often attacked, resulting in fin damages (Fig. 1). Significantly higher levels ($F_{(3, 15)} = 19.943$, $p < 0.05$) of caudal fin deterioration erosion were observed in females from groups NS48 and NS24 (23.8% ± 2.9% and 15.7% ± 3.5%, respectively). For both males and females in the rest of the groups, this value did not exceed a mean of 3% (Table 4). The experiment was stopped when the first female mortality occurred. The death was caused by the male attacking, and subsequently damaging, body tissue. After the end of the experiment, males and females were separated in different tanks for 2 weeks of the recovery period. No further mortality was observed.

Discussion

This study revealed differences in physiological response between pond-cultured males and females with regard to nest spawning under controlled conditions of the RAS.

The hematological and biochemical profiles are often used for estimation of physiological status and stress response in fish (Kusakabe et al. 2003; Noaksson et al. 2005; Ghosh and Joshi 2008; Westring et al. 2008; Milla et al. 2009; Sarameh et al. 2013; Falahatkar and Poursaeid 2014; Křišťan et al. 2014; Lepič et al. 2014; Svačina et al. 2016). The experimental design elucidated crucial points of pikeperch reproduction for both males and females, considering certain aspects of behavior, i.e., aggressive paternal behavior of males (Lappalainen et al. 2003), when fish were not separated after spawning completion. There is no investigation about this aspect in pikeperch paternal behavior with regard to the controlled condition of reproduction unit. Results from this study could serve for optimization of broodstock management procedures and for decreasing, or even eliminating, issues related to higher mortality of outdoor-reared broodstock and thus minimizing costs needed for production (Zakęś and Demska-Zakęś 2009; Zakęś et al. 2013).

In this study, strong and aggressive behavior of males drastically affected the physiological status of the females. Our results are in line with other authors reporting higher sensitivity of female pikeperch during reproduction (Rónyai 2007; Falahatkar and Poursaeid 2014; Falahatkar et al. 2014; Sarameh et al. 2013; Ljubobratović et al. 2017). Falahatkar and Poursaeid (2014) observed higher levels of cortisol in pikeperch females from control groups as well as in response to the hormonal induction compared to the males. The highest values were found in females treated with hCG. Similar pattern was found in our study, where both of the sexes were treated with hCG and females exhibited higher cortisol levels in blood compared to the males. However, due to the high variability, those differences were significant only in a control group. The baseline of cortisol concentrations was in range as described by Sarameh et al. (2013), although they were lower than those in juveniles reported by Falahatkar et al. (2012), which could be explained by the age of pikeperch used for the investigation.

Changes in blood plasma cortisol levels with regard to maturation are common in fish (Milla et al. 2009). Physiological changes occurring during the spawning period induced cortisol level fluctuation. Furthermore, the literature validates the essential role of corticosteroids for regulation of reproductive mechanisms (Cook et al. 1980; Pickering and Christie 1981; Bry 1985; Kime and Dolben 1985; Andersen 2002; Milla et al. 2009). In this study, higher cortisol level in the blood of females may not necessarily relate to stress alone but may stem from physiological changes in the final stages of maturation (Andersen 2002). Current fish literature reports a broad increase in blood cortisol levels during late maturation and spawning (Kusakabe et al. 2003; Noaksson et al. 2005; Westring et al. 2008; Milla et al. 2009; Faught and Vijayan 2018).

In response to physiological stress, cortisol released in fish body induces both glycogenesis and gluconeogenesis resulting in increased levels of GLU in the blood plasma which is directly related to the energy metabolism (Ghosh and Joshi 2008; Milla et al. 2009). This study observed consistently high levels of GLU in both males and females throughout the semi-controlled reproduction procedure. The only significant decrease of GLU was found in females from group SP24 that could indicate the positive effect of separation after spawning. Despite the absence of significance, there was an overall tendency of higher levels of GLU in groups NS24 and NS48 for both males and females. This could confirm that higher stress level was a consequence of keeping male and female together and concomitant male aggressive behavior (Lappalainen et al. 2003).

There is a lack of information regarding Ca^{2+} metabolism in fish; however, an increase in Ca^{2+} ion concentration is rather related to acute respiratory acidosis (Ghosh and Joshi 2008). This combined with lower numbers of erythrocytes could lead to insufficient nutrient transport and low tissue oxygenation with subsequent anemia (Nakayasu et al. 2002; Witeska 2015). In this study, levels of Ca^{2+} were significantly higher in males that were not separated from the females (NS48) after spawning and indicated higher movement activity in comparison to separated fish.

Physiological stress is often combined with increased movement activity of the fish (Thomas et al. 1999; Sarameh et al. 2013). Subsequent diminished oxygen availability leads to an increase of LACT in the blood plasma (Omlin and Weber 2010; Svačina et al. 2016). In this study, the higher LACT levels were found in males from group SP48, which is contradictory to our observations of intensive movement of the males due to aggressive behavior. The higher LACT level in separated males could be explained not only with increased movement activity but also with the stimulation of anaerobic glycolysis or the reduction of the lactate utilization rate (Omlin and Weber 2010). Apparently, concentration measurements of LACT in this study are not enough to fully reveal the changes in fish body while direct measurements of glycolytic flux would help to interpret results.

Significant differences were recorded in hematological parameters between fish that were separated after spawning and those kept together. In this study, significant changes of MCV were observed in both males and females which were not separated from each other after spawning. This difference might indicate an osmoregulatory failure of blood cells, leading to their deformation. Subsequently, insufficient function of these cells may lead to destruction of erythrocytes and concomitant anemia (Nakayasu et al. 2002; Gomułka et al. 2015; Witeska 2015).

During the experiment, males from groups NS24 and NS48 exhibited a significantly higher count of Leuko compare to groups SP24 and SP48. Our results are in line with Sarameh et al. (2013) who observed a high count of Leuko in pikeperch broodstock exposed to handling and different photoperiods. Observed changes of this parameter are more likely to be an outcome of the stress that fish experienced during reproduction. From the other hand, consistently high count of Leuko in female's plasma could be a result of a general fragility of their immune system during reproduction (Espelid et al. 1996; Bowden 2008).

Although there was no significant differences in Er count in females of any experimental group, the separation had a positive effect on this parameter, and 48 h after spawning, the numbers of Er count increased back to their at the beginning of the experiment (Table 2). This normalization period may represent approximate time required for broodstock to restore their physiological state.

Separation of females during semi-artificial reproduction under RAS conditions is a necessary step with regard to fish welfare practices. During the semi-artificial reproduction protocol, when limited space is available, females are often subjected to attack (Zakęś and Demska-Zakęś 2009). When both sexes are kept together, they are likely to exhibit skin damages caused by attacking, increasing vulnerability to infection and disease (Gomułka et al. 2007; Łuczyński et al. 2007; Rónyai 2007).

Despite the growing interest in pikeperch as a commercial fish species, there are only a few studies concerning behavioral monitoring in the context of artificial and semi-artificial reproduction (Drasovean and Blidariu 2013; Grozea et al. 2016; Baekelandt et al. 2019). Over the past decade, technology of digital recording has improved, significantly allowing behavioral monitoring of aquatic organism at lower cost (Huse and Skiftesvik 1990; Lucas and Baras

2000; Papadakis et al. 2012; Steen and Ski 2014). Although behavioral monitoring of fish is widely used in aquaculture, these studies are still rare for pikeperch (Drasovean and Blidariu 2013; Grozea et al. 2016). Hence, this study was focused on paternal behavior of pikeperch males and provided useful data necessary to understand the physiological changes occurring in the bodies of pikeperch broodstock. Photo analyses indicated much higher fin damage levels in females that were kept together with males after spawning compared to fish from other experimental groups (Fig. 1). The effect of male aggressive behavior was reflected in some of the hematological and biochemical parameters, while video recordings and photo analyses made clear a necessity of separating females after spawning in limited area.

Developing techniques for behavioral monitoring will help to improve semi-artificial reproduction protocol for pikeperch in terms of welfare. Remote monitoring systems are currently used for aquaculture (Steen and Ski 2014; Kuklina et al. 2018) and are useful in conditions when long-term observations are necessary or when human presence disturbs the animals. Pikeperch spawning is accompanied with intensive movement of the fish around the nest (Drasovean and Blidariu 2013). Using this system to determine intensive movements of fish in the tank could serve as an alarm indicating necessary human involvement, which, in case of this study, means separation of females immediately after spawning. Evaluation of fin erosion level was previously used in the assessment of rearing condition for pikeperch juveniles (Policar et al. 2016) and Eurasian perch (*Perca fluviatilis*; Stejskal et al. 2011) during intensive culture. Adámek et al. (2007) used the same principle of digital photo analysis to evaluate body injuries of fish that had escaped cormorant (*Phalacrocorax carbo sinensis*) attacks. The approach in this study permitted significant improvements of broodstock health status during reproduction and minimized, or even totally prevented, mortality after spawning.

Post-spawning losses of pikeperch broodstock is a common consequence of high sensitivity to manipulation and stress during reproduction (Gomułka et al. 2007; Łuczyński et al. 2007; Rónyai 2007; Zakęś and Demska-Zakęś 2009). Cortisol released in to the bloodstream demonstrates immunosuppressive effect resulting in low effectivity of the immune system (Witeska 2015). Without application of special treatments, subsequent mortality of broodstock could reach 100% in several days after spawning (Policar et al. 2019). In this study, experimental trials were stopped after occurrence of first mortality. A female died after being kept with male for 48 h in one tank. Male's protective behavior to the nest is well known, and in limited space, females are often targeted which leads to various injures and a decrease in health status (Zakęś and Demska-Zakęś 2009; Policar et al. 2019). In this study, relatively small-volume tanks were used for the reproduction (350 l). It may be important to understand how the volume of water and subsequent available space can affect aggressive behavior in male pikeperch for a managerial point of view. Further research is needed to investigate the size of the territory guarded by male pikeperch to enhance our understanding of reproduction techniques and thereby improve broodstock management practices during reproduction in controlled conditions.

Hematological and biochemical alterations are useful indicators for evaluating fish physiological status; however, among pikeperch, these indices vary depending on age, maturation, and general health status (Falahatkar et al. 2012; Křišťan et al. 2012; Sarameh et al. 2013; Falahatkar and Poursaeid 2014). This study contributed to our understanding of semi-artificial reproduction of pikeperch broodstock caught from natural environment, when fish are continually exposed to multiple stressors. Broodstock behavior monitoring allowed us to minimize mortality after spawning by separating females, thereby significantly improving their physiological status during reproduction. Semi-artificial reproduction is an effective and widely used

protocol in pikeperch aquaculture (Blecha et al. 2016; Malinovskyi et al. 2018). This method of reproduction results in high spawning success of broodstock and improved egg incubation, thereby improving the hatching rate by about 72% (Blecha et al. 2016). Further investigations focusing on physiological changes will improve our understanding of the stress response and risks associated with reproduction. Investigation of ecological and behavioral features of pikeperch species could be used to minimize negative consequences of reproduction in captivity, including reducing broodstock mortality, and may dramatically improve the effectiveness of broodstock management procedures.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethic statement The study performed and the experimental broodstock handled were in accordance with national and international guidelines for the protection of animal welfare (EU-harmonized Animal Welfare Act of the Czech Republic). The experimental unit is licensed (No. 2293/2015-MZE-17214 and No. 55187/2016-MZE-17214 within the project NAZV QK1710310) according to the Czech National Directive (the Law against Animal Cruelty, No. 246/1992).

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CHAPTER 4

POST-SPAWNING BATH TREATMENTS TO REDUCE MORBIDITY AND MORTALITY OF POND-CULTURED PIKEPERCH (*SANDER LUCIOPERCA* L.) BROODSTOCK

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EUROPEAN PERCID FISH CULTURE

Post-spawning bath treatments to reduce morbidity and mortality of pond-cultured pikeperch (*Sander lucioperca* L.) broodstock

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Abstract
High mortality (88.9–91.4%) of pond-cultured pikeperch *Sander lucioperca* broodstock was found 10 days post-spawning primarily due to body injury (15.2–18.2% of body area) and secondary fungal infection (27.6–34.8% of body area). Ninety days post-spawning mortality was 97.1–98.1%. Surviving broodstock showed satisfactory condition with no observable pathology. Thirty-six treatments were evaluated for effects on post-spawning injury, fungal infection, and broodstock mortality: salt short (60–240 min) and long baths (36–144 h) at concentrations of 5–20 g L^{-1} and 2.5–10 g L^{-1}, respectively, and formalin short (15–60 min) and long baths (24–72 h) at 0.15–0.30 ml L^{-1} and 0.015–0.030 ml L^{-1}, respectively. The treatments most effective in reducing post-spawning pathology and mortality were salt baths for 144 h at 2.5, 5, and 10 g L^{-1} and formalin at 0.015 ml L^{-1} for 48 h. These treatments were evaluated in the following trial, and their positive effect was confirmed by low mortality of broodstock (0–8.3%) and low (3.0–10.5%) injury at 10 days post-spawning and no additional mortality or body injury at 90 days post-spawning. No secondary fungal infection was observed at either time post-spawning. The three salt baths are recommended as effective treatments for reduction of post-spawning morbidity and mortality of pond-cultured pikeperch broodstock.

Keywords Formalin · Fungi · Reproduction · Secondary infection · Salt · *Sander lucioperca* · Skin lesion · Welfare

Introduction

Pikeperch is a valuable commercial species (Blecha et al. 2015) providing diversification for European inland aquaculture (Policar and Adámek 2013) through pond and intensive fish

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farming (Policar et al. 2016). Its intensive culture in recirculating aquaculture systems (RAS) has been practiced for more than 15 years, mainly in Western and Central Europe (Policar et al. 2013). Pond culture and commercial fishing in natural open waters are common in Eastern Europe and Asia (Steenfeldt et al. 2015). Pikeperch is under pressure from commercial fishing, and wild populations is declining in many countries (Dil 2008; Falahatkar et al. 2018). Currently, only RAS alone or combined with pond culture provides high-quality, stable, and sustainable production of pikeperch for restocking and market (Steenfeldt et al. 2015; Policar et al. 2016; Falahatkar et al. 2018).

The optimization of broodstock management and reproduction are critical in ensuring healthy mature broodstock-producing larvae for culture (Fontaine et al. 2015; Overton et al. 2015). Nest spawning, also known as semi-artificial spawning, under controlled conditions is an effective method to provide stable pikeperch larval production (Blecha et al. 2016a; Malinovskyi et al. 2018). However, the environment manipulation, hormone treatment, fish handling, and nest spawning or stripping in controlled reproduction can increase stress (Falahatkar and Samaneh 2014) and affect the immune system and hematology of pikeperch broodstock (Sarameh et al. 2012, 2013). Broodstock can be injured by aggressive sex-related behaviour (Rónyai 2007) and handling and incur secondary fungal infection (Demska-Zakes and Zakes 2002; Zakęś and Demska-Zakęś 2009).

Broodstock mortality in both sexes occurs during and after the reproductive period, with rates differing between males and females, depending on latency in female (Zakęś and Demska-Zakęś 2009), spawning procedures (Rónyai 2007; Zakęś and Demska-Zakęś 2009; Policar et al. 2011a; Zakęś et al. 2013), fish origin (Łuczyński et al. 2007; Zakęś and Demska-Zakęś 2009; Zakęś et al. 2013), use of anesthetics (Kristan et al. 2012, 2014), and application of preventive treatments during or following handling and spawning (Gomułka et al. 2007).

The aim of this study was to assess broodstock mortality during and after nest spawning and to assess selected post-spawning treatments with the aim of effectively reducing broodstock injury, morbidity, and mortality in pond-cultured pikeperch.

Materials and methods

Broodstock management and nest spawning

Mature pond-cultured pikeperch broodstock (total length TL = 548 ± 57 mm and body weight $W = 1452 \pm 275$ g, age 3–4 years), 1:1 sex ratio, were used for nest spawning every year from 2009 to 2015. Broodstock were captured from polyculture ponds of production company Rybářství Nové Hrady Ltd. during the spring harvest season, transferred to the University of South Bohemia, Faculty of Fisheries and Protection of Waters (USB, FFPW), and stocked into a 10 × 5 × 1-m earthen pond. Fish were provided with prey, roach *Rutilus rutilus*, and topmouth gudgeon *Pseudorasbora parva* (TL = 40–70 mm), six prey fish per broodfish per day.

Each year, broodstock were selected for nest spawning as follows: females with stage 3 oocytes identified according to Żarski et al. (2012) and males spontaneously releasing sperm. Both sexes were hormone treated with hCG (Chorulon) at 500 IU per kg (Kristan et al. 2013; Blecha et al. 2016a) before stocking in pairs into 350-L tanks of RAS with water temperature (WT) 13.5 ± 0.8 °C; $O_2 = 101 \pm 13.5\%$; and light regime 14 L:10D, 100 lx. Artificial turf, fiber length 35 mm, as spawning substrate was installed on the bottom of each tank (Malinovskyi

et al. 2018). Fish were left undisturbed for the subsequent 60 h. After this period, nests were checked every 2 h for the presence of fertilized eggs. The short interval was used to allow intervention to prevent injury to the female by aggressive male behavior after spawning. Immediately after spawning, broodfish were removed from the tank for further investigation. Eggs were incubated in the tanks under RAS conditions until hatching following Blecha et al. (2016a) and Malinovskyi et al. (2018).

Phase 1: post-spawning broodstock morbidity and mortality without treatment

Thirty-five pairs of pikeperch broodstock were spawned in each year from 2009 to 2011, totaling 105 pairs during 3 years. After spawning, body weight, W_1 in g, and total length, TL_1 in mm, were found according to Policar et al. (2011b) and all surviving fish were photographed under anesthesia (clove oil 0.03 ml L^{-1}). Fulton's coefficient (FC_1) = 100 W_1/TL_1^3 (Policar et al. 2011b) and mortality rate ((M_1 in %) = (dead fish/all fish)100) were calculated for both sexes. Extent of skin injury was calculated from photograph with QuickPHOTO MICRO 3.2 image analysis as percentage of injured body area (IBA_1%) and fungal infection area (FIA_1%) of total body surface according to Adámek et al. (2007). Fish were also marked with a ventral fin clip (female left and male right fin) for future identification according to Blecha et al. (2016b), and after 1 h, acclimatization returned to the earthen pond (WT = 16.1 ± 0.8 °C; O_2 = 98 ± 18.1%). Broodstock was daily supplied there with six prey per broodfish per day. The pond was harvested after 10 days. Dead and surviving broodstock were counted for the determination of cumulative mortality rate (M_2%), photographed for evaluation of IBA_2% and FIA_2%, and measured (TL_2) and weighed (W_2) for calculation of FC_2. Surviving fish were stocked into a larger experimental pond at USB FFPW (0.1 ha; WT = 21.1 ± 2.8 °C; O_2 = 89 ± 20.8%) with approximately six prey fish per broodfish per day. After 80 days, the fish were harvested, and surviving fish were identified by sex, counted, measured (TL_3) and weighed (W_3), and final cumulative mortality rate (M_3%) with FC_3, IBA_3%, and FIA_3% were calculated as 90 days post-spawning.

Phase 2: preliminary evaluation of post-spawning treatment to reduce broodstock morbidity and mortality

Over the 3 years (2009–2011), high post-spawning broodstock mortality was confirmed. In 2012 and 2013, two antifungal treatments common used in aquaculture, salt, (Sűdwestdeutsche Salzwerke AG, Germany) and formalin 37.5% (PENTA Ltd., Czech Republic) according to Rodger and Phelps (2015), Kouba et al. (2010), and Policar et al. (2011c) were tested as potential tools for the reduction of post-spawning broodstock mortality. Both chemicals were administered in short (SB) and long baths (LB) at the following concentrations and durations: salt SB at 5, 10, and 15 g L^{-1} for 60, 120, and 240 min; salt LB at 2.5, 5, and 10 g L^{-1} for 36, 72, and 144 h; and formalin SB at 0.15, 0.20, and 0.30 ml L^{-1} for 15, 30, and 60 min; and LB at 0.015, 0.020, and 0.030 ml L^{-1} for 24, 48, and 72 h. Fish were held under RAS conditions as in Phase 1. A total of 36 treatments were tested, each in one breeding pair per year immediately after the nest spawning. Hence, over the course of two years, each treatment was tested in four fish. Each year, following spawning of 50 pairs, mortality rate without regard to sex was calculated, and mean mortality (M_1) of both years was calculated. The selected 36 surviving pairs as experimental fish were measured, weighed, and photographed for identification of initial values of TL_1, W_1, FC_1, IBA_1, and FIA_1. Fish were also marked with the combination of the clip of ventral (right-R or left-L), pectoral (R or L), first or second dorsal fin, or

no clip and the application of visible implant elastomer (VIE) tag (Northwest Marine Technology, Inc.; USA) (pink, orange, red, yellow, green, or no tag) for later identification of each treatment. After treatment, all surviving fish of all groups were stocked into the same earthen pond as Phase 1. Ten days post-spawning, the pond was harvested. Dead and surviving fish were counted, photographed, measured, and weighed for M_2, TL_2, W_2, FC_2, IBA_2, and FIA_2. Surviving fish were stocked into a large pond for an 80-day culture period under conditions of Phase 1, after which, final characteristics were assessed for each group (M_3, W_3, TL_3, FC_3, IBA_3, FIA_3). The most effective treatments were selected for the following trial.

Phase 3: trial of selected treatments for the reduction of post-spawning broodstock morbidity and mortality

In 2014 and 2015, every year, 50 pairs of pikeperch broodstock were nest spawned in separate tanks. Mortality rate after spawning (M_1) was calculated, and 36 surviving pairs as experimental fish were measured, weighed, and photographed for W_1, TL_1, FC_1, IBA_1, and FIA_1. The post-spawning test consisted of four long bath treatments comprising three salt concentrations ($Salt_{1–3}$) 2.5, 5, and 10 g L^{-1} for 144 h and one formalin (Form) at a concentration of 0.015 ml L^{-1} for 48 h, and was tested in 24 broodstock pairs immediately after spawning. Trial included two control groups: RW, broodstock kept in flow-through water system the River Blanice without treatment, and RAS, broodstock kept under RAS conditions similar to those of the experimental groups with no treatment. Each experimental and control group comprised 6 males and 6 females kept under previously described conditions until 10 days post-spawning. Males were marked on the right side and females on the left with VIE tags (pink, orange, red, or yellow) for later identification of treatment and sex. Parameters M_2, TL_2, W_2, FC_2, IBA_2, and FIA_2 were assessed in all fish. Surviving fish were stocked into a single large pond for 80-day culture as in Phases 1 and 2. The parameters M_3, W_3, TL_3, FC_3, IBA_3, and FIA_3 were found, calculated, and compared among tested treatments with respect to sex after this culture. Each year was considered a repetition of the experimental period.

Statistical analysis

All obtained data are expressed as mean ± SD of three- (Phase1: 2009–2011) or two-year repetitions (Phase 2: 2012 and 2013 and Phase 3: 2014 and 2015). All data from Phase 1 and IBA and FIA in Phase 2 were analyzed using one-way analysis of variance (ANOVA) and Tukey's post-test (post-spawning mortality relative to sex in Phase 1 and to treatment in Phase 2). Data from Phase 3 were compared with two-way ANOVA and Tukey's post-test (post-spawning mortality relative to post-spawning treatment and sex). Statistical assessment was performed by STATISTICA 6.1 (StatSoft, Inc. Czech Republic) with $P < 0.05$ as the level of significance.

Results

Phase 1: post-spawning broodstock morbidity and mortality without treatment

Low broodstock mortality ($M_1 = 2.9–6.7\%$) was found immediately after spawning during Phase 1 of this study. Mortality rate of females was 6.7 ± 3.06% compared with 2.9 ± 0.95% for males. Both sexes showed $IBA_1 = 16.5–19.8\%$. Females exhibited $FIA_1 = 5.8 \pm 2.7\%$

compared with no fungal infection in males, likely the source of higher female mortality in this phase. Lower FC_1 of females (0.8 ± 0.12) compared with males ($FC_1 = 0.9 \pm 0.05$) was probably an effect of ovulation and spawning. Ten days post-spawning, high mortality of both sexes was found ($M_2 = 88.9–91.4\%$) with no differences between sexes, and significantly increased fungal infection compared with FIA_1 was evident in both sexes ($FIA_2 = 27.6–34.8\%$). On the contrary, body injury was not increased at 10 days post-spawning in either sex ($IBA_2 = 15.2–18.2\%$) and was similar to IBA_1 values. Fungal infection secondary to skin injury was most likely the primary cause of broodstock morality. FC_2 of surviving females reached 0.9, similar to that of males. After following 80-day culture, broodstock mortality (M_3) reached 97.1–98.1% in both sexes. The three surviving females and two males from the initial 105 pairs showed satisfactory condition ($FC_3 = 0.9–1.1$) with a higher value in females and no injuries or fungal infection (Table 1).

Phase 2: preliminary evaluation of post-spawning treatments to reduce broodstock morbidity and mortality

Broodstock mortality ($M_1 = 5.0 \pm 1.0\%$), fish condition ($FC_1 = 0.8–0.9$), injury ($IBA_1 = 17.5 \pm 12.5\%$), and fungal infection ($FIA_1 = 2.0 \pm 1.0\%$) immediately post-spawning was similar to that observed in Phase 1.

Total (100%) broodstock mortality and low FC_2 (0.8) were 10 days post-spawning in most short salt baths with the exception of 120 and 240-min exposure to the highest salt concentration (15 g L^{-1}), for which, there was 75% mortality. Long bath salt treatments were effective in reducing broodstock mortality. Exposure to salt concentration 2.5 g L^{-1} for 36 h was associated with 75% mortality rate and FC_2 of 0.8. Concentrations of 5 and 10 g L^{-1} for 36 h, as well as 2.5 and 5 g L^{-1} for 72 h, showed lower mortality of 25–50% and FC_2 of 0.9–1.0. No mortality was observed with the highest salt concentration (10 g L^{-1}) with 72-h exposure and all salt concentrations for 144 h. Also, good fish condition was found ($FC_2 = 0.9–1.0$) after the mentioned treatments. These most effective salt baths were associated with $IBA_2 = 5.0–6.0\%$ and $FIA_2 = 0\%$. Salt baths with lower effectiveness did not significantly reduce broodstock mortality (100–75%), and the fish showed higher IBA_2 (15.1–18.4%) and FIA_2 (5.0–30.5%) (Table 2). Groups exposed to the 144-h salt bath at 5 and 10 g L^{-1} also showed zero mortality at 90 days post-spawning. The 2.5 g L^{-1} exposure for 144 h and 10 g L^{-1} for 72 h also produced acceptable mortality of 25% at 90 days post-spawning. The mortality primarily occurred during the following 80-day culture and may have been unrelated to spawning. After an 80-day pond culture, all surviving broodstock showed higher FC_3 (1.0–1.1) compared with FC_2 (0.8–1.0). All fish were without body injury or fungal infection (Table 2). Salt baths at 2.5, 5, and 10 g L^{-1} for 144 h were selected as the most effective treatments for the following trial.

The formalin exposures at 0.15–0.30 ml L^{-1} for 15–60 min were associated with broodstock mortality of 100% at 10 days post-spawning. Source of the mortality was probably injury ($IBA_2 = 16.5–18.7\%$), fungal infection ($FIA_2 = 27.9–35.2\%$), and low fish condition ($FC_2 = 0.8$). Exposure to formalin at 0.015–0.030 ml L^{-1} for 24 h decreased mortality to 25% and showed decreased IBA_2 (7.3–10.5%) and FIA_2 (0–2.0%) and increased fish condition ($FC_2 = 0.9$) compared with short formalin baths. Formalin baths for 48–72 h at all tested concentrations were associated with no mortality, body injury area of 4.5–5.9%, no observation of fungal infection, and increased fish condition ($FC_2 = 1.0$). Pikeperch broodstock treated with long formalin baths had also showed a low mortality rate ($M_3 = 0–25\%$) after an 80-day pond culture (Table 3). The lowest concentration of 0.015 ml L^{-1} with 48 h exposure was selected as the most effective treatment for the following trial.

Table 1 Mortality rate (M_{1-3}), fish condition (FC_{1-3}), and percentages of injured body (IBA_{1-3}) and fungal infection area (FIA_{1-3}) of pikeperch broodstock immediately after spawning, at 10 and 90 days post-spawning. Data are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences between sexes ($P < 0.05$)

Parameter	After spawning				10 days post-spawning				90 days post-spawning			
	M_1 (%)	FC_1	IBA_1 (%)	FIA_1 (%)	M_2 (%)	FC_2	IBA_2 (%)	FIA_2 (%)	M_3 (%)	FC_3	IBA_3 (%)	FIA_3 (%)
Female ($n = 105$)	6.7 ± 3.06^{b}	0.8 ± 0.12^{a}	19.8 ± 14.95^{a}	5.8 ± 2.7^{b}	91.4 ± 5.2^{a}	0.9 ± 0.1^{a}	18.2 ± 17.8^{a}	34.8 ± 25.4^{b}	97.1 ± 1.4^{a}	1.1 ± 0.1^{b}	0 ± 0^{a}	0 ± 0^{a}
Male ($n = 105$)	2.9 ± 0.95^{a}	0.9 ± 0.05^{b}	16.5 ± 7.75^{a}	0 ± 0^{a}	88.9 ± 5.8^{a}	0.9 ± 0.05^{b}	15.2 ± 7.7^{a}	27.6 ± 24.5^{a}	98.1 ± 0.95^{a}	0.9 ± 0.05^{a}	0 ± 0^{a}	0 ± 0^{a}

Table 2 Mortality rate ($M_{2–3}$), fish condition ($FC_{2–3}$), and percentage of injured body area ($IBA_{2–3}$) and fungal infection area ($FIA_{2–3}$) of pikeperch broodstock at 10 and 90 days post-spawning after short (SB) and long (LB) salt bath. SB concentrations 5, 10, and 15 g L^{-1} with exposure time 60, 120, and 240 min; LB concentrations 2.5, 5, and 10 g with exposure 36, 72, and 144 h. Data are expressed as mean ± standard deviation. Different letters in the column of IBA_2 and FIA_2 indicate significant differences among treatments ($P < 0.05$)

Treatment ($n = 4$)	10 days post-spawning				90 days post-spawning			
Concentration/ exposure	M_2 (%)	FC_2	IBA_2 (%)	FIA_2 (%)	M_3 (%)	FC_3	IBA_3 (%)	FIA_3 (%)
SB 5 g L^{-1}/60 min	100 ± 0	0.8 ± 0.13	17.5 ± 9.9^b	28.4 ± 22.5^c	100 ± 0	–	–	–
SB 10 g L^{-1}/60 min	100 ± 0	0.8 ± 0.12	18.4 ± 10.7^b	30.2 ± 27.5^c	100 ± 0	–	–	–
SB 15 g L^{-1}/60 min	100 ± 0	0.8 ± 0.11	16.8 ± 10.1^b	25.4 ± 21.8^c	100 ± 0	–	–	–
SB 5 g L^{-1}/120 min	100 ± 0	0.8 ± 0.15	16.5 ± 9.8^b	30.5 ± 24.8^c	100 ± 0	–	–	–
SB 10 g L^{-1}/120 min	100 ± 0	0.8 ± 0.10	16.7 ± 9.9^b	28.7 ± 24.5^c	100 ± 0	–	–	–
SB 15 g L^{-1}/120 min	75 ± 25	0.8 ± 0.13	16.2 ± 10.3^b	25.7 ± 23.5^c	75 ± 25	1.1	0 0 ± 0	0 ± 0
SB 5 g L^{-1}/240 min	100 ± 0	0.8 ± 0.15	15.5 ± 9.1^b	24.8 ± 22.5^c	100 ± 0	–	–	–
SB 10 g L^{-1}/240 min	100 ± 0	0.8 ± 0.11	15.2 ± 10.0^b	27.9 ± 24.5^c	100 ± 0	–	–	–
SB 15 g L^{-1}/240 min	75 ± 25	0.8 ± 0.12	15.1 ± 9.8^b	8.9 ± 7.5^b	100 ± 0	–	–	–
LB 2.5 g L^{-1}/36 h	75 ± 25	0.8 ± 0.14	15.5 ± 9.5^b	5.0 ± 4.2^b	75 ± 25	1.0	0 0 ± 0	0 ± 0
LB 5 g L^{-1}/36 h	50 ± 0	0.9 ± 0.13	7.5 ± 5.5^a	5.5 ± 5.0^b	50 ± 0	1.1 ± 0.10	0 0 ± 0	0 ± 0
LB 10 g L^{-1}/36 h	25 ± 0	0.9 ± 0.12	7.8 ± 5.5^a	5.0 ± 2.0^b	50 ± 0	1.0 ± 0.10	0 0 ± 0	0 ± 0
LB 2.5 g L^{-1}/72 h	50 ± 50	1.0 ± 0.14	6.8 ± 5.3^a	4.7 ± 4.0^b	50 ± 0	1.1 ± 0.10	0 0 ± 0	0 ± 0
LB 5 g L^{-1}/72 h	25 ± 25	0.9 ± 0.14	7.5 ± 5.5^a	5.0 ± 1.5^b	50 ± 0	1.0 ± 0.10	0 0 ± 0	0 ± 0
LB 10 g L^{-1}/72 h	0 ± 0	1.0 ± 0.11	6.0 ± 2.0^a	0 ± 0^a	25 ± 0	1.1 ± 0.10	0 0 ± 0	0 ± 0
LB 2.5 g L^{-1}/144 h	0 ± 0	0.9 ± 0.12	5.0 ± 2.0^a	0 ± 0^a	25 ± 0	1.0 ± 0.10	0 0 ± 0	0 ± 0
LB 5 g L^{-1}/144 h	0 ± 0	1.0 ± 0.12	5.5 ± 1.9^a	0 ± 0^a	0 ± 0	1.1 ± 0.10	0 0 ± 0	0 ± 0
LB 10 g L^{-1}/144 h	0 ± 0	1.0 ± 0.13	5.7 ± 1.8^a	0 ± 0^a	0 ± 0	1.1 ± 0.10	0 0 ± 0	0 ± 0

Phase 3: trial of selected treatments for the reduction of post-spawning broodstock morbidity and mortality

Prior to Phase 3, broodstock mortality ($M_1 = 3.0 \pm 1.0\%$), fish condition ($FC_1 = 0.8–0.9$), injury ($IBA_1 = 16.7 \pm 13.2\%$), and fungal infection ($FIA_1 = 1.8 \pm 1.2\%$) were similar to that observed in previous phases directly after nest spawning.

The highest mortality rate was found in both sexes ($M_2 = 83.3–91.7\%$) from group RW at 10 days post-spawning as well as the highest injury ($IBA_1 = 15.2–18.9\%$) and fungal infection

Table 3 Mortality rate ($M_{2–3}$), fish condition (FC_{2-3}), and percentage of injured body ($IBA_{2–3}$) and fungal infection area ($FIA_{2–3}$) of pikeperch broodstock at 10 and 90 days post-spawning after short (SB) and long (LB) formaldehyde baths. SB concentrations 0.15, 0.20, and 0.30 ml L^{-1} with exposure 15, 30, and 60 min; LB concentrations 0.015, 0.020, and 0.030 ml L^{-1} with exposure 24, 48, and 72 h). Data are expressed as mean ± standard deviation. Different letters in the column of IBA_2 and FIA_2 indicate significant differences among treatments ($P < 0.05$)

Treatment ($n = 4$)	10 days post-spawning				90 days post-spawning			
Concentration/ exposure	M_2 (%)	FC_2	IBA_2 (%)	FIA_2 (%)	M_3 (%)	FC_3	IBA_3 (%)	FIA_3 (%)
SB 0.15 ml L^{-1}/15 min	100 ± 0	0.8 ± 0.14	18.5 ± 6.9^{b}	35.2 ± 29.1^{b}	100 ± 0	–	–	–
SB 0.20 ml L^{-1}/15 min	100 ± 0	0.8 ± 0.11	17.9 ± 7.9^{b}	32.1 ± 25.4^{b}	100 ± 0	–	–	–
SB 0.30 ml L^{-1}/15 min	100 ± 0	0.8 ± 0.11	18.7 ± 7.2^{b}	28.5 ± 27.8^{b}	100 ± 0	–	–	–
SB 0.15 ml L^{-1}/30 min	100 ± 0	0.8 ± 0.15	17.9 ± 7.5^{b}	31.5 ± 27.8^{b}	100 ± 0	–	–	–
SB 0.20 ml L^{-1}/30 min	100 ± 0	0.8 ± 0.10	17.1 ± 6.9^{b}	30.7 ± 27.5^{b}	100 ± 0	–	–	–
SB 0.30 ml L^{-1}/30 min	100 ± 0	0.8 ± 0.13	17.5 ± 7.3^{b}	35.7 ± 24.4^{b}	100 ± 0	–	–	–
SB 0.15 ml L^{-1}/60 min	100 ± 0	0.8 ± 0.15	16.9 ± 6.2^{b}	27.9 ± 24.3^{b}	100 ± 0	–	–	–
SB 0.20 ml L^{-1}/60 min	100 ± 0	0.8 ± 0.11	16.5 ± 7.0^{b}	28.9 ± 26.5^{b}	100 ± 0	–	–	–
SB 0.30 ml L^{-1}/60 min	100 ± 0	0.8 ± 0.11	16.8 ± 6.2^{b}	27.9 ± 24.5^{b}	100 ± 0	–	–	–
LB 0.015 ml L^{-1}/24 h	25 ± 25	0.9 ± 0.14	10.5 ± 3.5^{ab}	2.0 ± 1.7^{a}	50 ± 50	1.0	0 ± 0	0 ± 0
LB 0.020 ml L^{-1}/24 h	25 ± 25	0.9 ± 0.13	7.3 ± 0.8^{a}	1.5 ± 1.0^{a}	50 ± 0	1.1 ± 0.10	0 ± 0	0 ± 0
LB 0.030 ml L^{-1}/24 h	25 ± 25	0.9 ± 0.12	7.7 ± 0.5^{a}	0 ± 0^{a}	50 ± 0	1.0 ± 0.10	0 ± 0	0 ± 0
LB 0.015 ml L^{-1}/48 h	0 ± 0	1.0 ± 0.14	5.8 ± 1.3^{a}	0 ± 0^{a}	25 ± 0	1.1 ± 0.10	0 ± 0	0 ± 0
LB 0.020 ml L^{-1}/48 h	0 ± 0	1.0 ± 0.14	5.9 ± 0.5^{a}	0 ± 0^{a}	25 ± 0	1.0 ± 0.10	0 ± 0	0 ± 0
LB 0.030 ml L^{-1}/48 h	0 ± 0	1.0 ± 0.11	5.6 ± 2.0^{a}	0 ± 0^{a}	25 ± 0	1.0 ± 0.10	0 ± 0	0 ± 0
LB 0.015 ml L^{-1}/72 h	0 ± 0	1.0 ± 0.12	5.0 ± 2.0^{a}	0 ± 0^{a}	0 ± 0	1.0 ± 0.10	0 ± 0	0 ± 0
LB 0.020 ml L^{-1}/72 h	0 ± 0	1.0 ± 0.12	4.5 ± 1.9^{a}	0 ± 0^{a}	0 ± 0	1.1 ± 0.10	0 ± 0	0 ± 0
LB 0.030 ml L^{-1}/72 h	0 ± 0	1.0 ± 0.13	4.7 ± 1.8^{a}	0 ± 0^{a}	0 ± 0	1.1 ± 0.10	0 ± 0	0 ± 0

(FIA_1 = 30.1–35.6%). Lower, but unacceptable, mortality was observed in females (M_2 = 75.0 ± 8.3%) and males (M_2 = 50.0 ± 16.7%) of the RAS group, with high injury (IBA_1 = 13.2–14.2%) and fungal infection (FIA_1 = 23.1–28.7%). Significantly lower mortality rates were found with all tested salt and formalin treatments. No mortality was seen in fish exposed to the highest salt and formalin concentrations. In the other two salt groups, males showed zero mortality and females 8.3 ± 8.3% at 10 days post-spawning. No mortality was observed with any tested salt and formalin treatment during the 80-day pond culture. Surviving females had

higher FC_3 (1.1 ± 0.1) compared with males (0.9 ± 0.1), and no fish exhibited body injury or fungal infection. Females in the RW and RAS groups and males in the RW group had the highest mortality after the 80-day pond culture, reaching 91.7–100%. Males from the RAS group had mortality 66.7 ± 16.7 (Table 4).

Discussion

Pond-cultured pikeperch broodstock have been effectively used for reproduction in most European countries, particularly, in Central and Eastern Europe where pond aquaculture is traditional (Schlumberger and Proteau 1996; Łuczyński et al. 2007; Steenfeldt et al. 2015; Falahatkar et al. 2018). Authors of this study have used this kind of broodstock for reproduction from 2009 till today not only for experimental work (Policar et al. 2011a; Blecha et al. 2015, 2016a, b, c; Samarin et al. 2015; Kristan et al. 2013, 2018) but also for commercial production of pikeperch larvae and juveniles. Generally, pond-cultured percid broodstock have higher spawning success and better gonad development (GSI) and production of sex steroids compared with domesticated fish (Křišťan et al. 2012; Khendek et al. 2017, 2018). Domesticated or RAS-cultured pikeperches present lower sensitivity to manipulation and handling (Łuczyński et al. 2007; Zakęś and Demska-Zakęś 2009; Zakęś et al. 2013; Ljubobratović et al. 2017) and better adaptation to chronic handling stress (Douxfils et al. 2015), leading to lower broodstock injury and post-spawning mortality (Rónyai 2007; Zakęś and Demska-Zakęś 2009; Křišťan et al. 2012; Ljubobratović et al. 2017). Pikeperch broodstock cultured in RAS is expensive and very demanding on professional skills, optimal photo-thermal, and feeding regime (Fontaine et al. 2015; Kestemont and Henrotte 2015; Ljubobratović et al. 2017). Production of broodstock with pond culture is less costly, but its efficacy is negatively affected by droughts, floods, predation, poaching, insufficient prey supply, and other unpredictable factors (Policar et al. 2011a). Broodstock management is the most important phase in aquaculture, as it provides the basis for the following production steps (Fontaine et al. 2015). Broodstock management should be optimized concerning maturation stage, spawning, and acceptable handling (Fontaine et al. 2015; Żarski et al. 2015) with an effort to reduce stress, morbidity, and finally, mortality pikeperch broodstock during spawning and post-spawning period (Gomułka et al. 2007; Łuczyński et al. 2007; Rónyai 2007). The reusing of broodstock for the next reproduction cycle is key action of effective and profitable pikeperch farming where pond-cultured brood fish have been used (Zakęś and Demska-Zakęś 2009; Policar et al. 2011a; Zakęś et al. 2013).

Stress response of both sexes in pikeperch broodstock on different photoperiods, hormonal treatment, and handling was well described by Sarameh et al. (2012, 2013) and Falahatkar and Samaneh (2014) during a reproductive season. This current study did not observe exact stress response of pikeperch broodstock. Broodstock apparent manifestations of spawning physiology and injury such as level of body injuries and intensity of secondary fungal infection were monitored, and their negative effect on broodstock survival was tried to be reduced in this study.

Sensitivity of wild and pond-cultured pikeperch broodstock to stress and high mortality several days post-spawning, with mortality rates of 31.1–100%, has been reported (Gomułka et al. 2007; Łuczyński et al. 2007; Rónyai 2007; Zakęś and Demska-Zakęś 2009). This study found similar high mortality of pikeperch broodstock 10 days post-spawning (50–100%) with no and in tested ineffective post-spawning treatments.

Table 4 Mortality rate ($M_{2–3}$), fish condition ($FC_{2–3}$), and percentage of injured body ($IBA_{2–3}$) and fungal infection area ($FIA_{2–3}$) of pikeperch broodstock at 10 and 90 days post-spawning. Salt concentrations ($Salt_{1–3}$) 2.5, 5, and 10 g L^{-1} with exposure duration 144 h and formaldehyde concentration (Form) 0.015 ml L^{-1} with exposure duration 48 h compared with two control groups: RW water flow-through system with River Blanice water and RAS with no chemical treatment. Data are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences among treatments and sexes ($P < 0.05$)

Treatment	Sex ($n = 12$)	10 days post-spawning				90 days post-spawning			
		M_2 (%)	FC_2	IBA_2 (%)	FIA_2 (%)	M_3 (%)	FC_3	IBA_3 (%)	FIA_3 (%)
RW	F	91.7 ± 8.4^{e}	0.8 ± 0.1^{a}	18.9 ± 7.4^{c}	35.6 ± 18.9^{d}	100 ± 0^{e}	–	–	–
	M	83.3 ± 16.7^{e}	0.9 ± 0.1^{a}	15.2 ± 7.4^{b}	30.1 ± 15.9^{d}	91.7 ± 8.3^{d}	0.9^{a}	0 ± 0^{a}	0 ± 0^{a}
RAS	F	75.0 ± 8.3^{d}	0.8 ± 0.1^{a}	14.2 ± 7.4^{b}	28.7 ± 8.7^{c}	91.7 ± 8.3^{d}	1.0^{ab}	0 ± 0^{a}	0 ± 0^{a}
	M	50 ± 16.7^{c}	0.9 ± 0.1^{a}	13.2 ± 7.4^{b}	23.1 ± 6.0^{c}	66.7 ± 16.7^{c}	0.9 ± 0.1^{a}	0 ± 0^{a}	0 ± 0^{a}
Salt	F	8.3 ± 8.3^{b}	0.9 ± 0.1^{a}	10.5 ± 5.4^{b}	5.1 ± 2.0^{b}	8.3 ± 8.3^{b}	1.1 ± 0.1^{b}	0 ± 0^{a}	0 ± 0^{a}
2.5 g L^{-1}/144 h	M	0 ± 0^{a}	0.9 ± 0.1^{a}	8.4 ± 4.4^{b}	5.1 ± 2.0^{b}	0 ± 0^{a}	0.9 ± 0.1^{a}	0 ± 0^{a}	0 ± 0^{a}
Salt	F	8.3 ± 8.3^{b}	0.9 ± 0.1^{a}	6.5 ± 4.7^{ab}	0 ± 0^{a}	8.3 ± 8.3^{b}	1.1 ± 0.1^{b}	0 ± 0^{a}	0 ± 0^{a}
5 g L^{-1}/144 h	M	0 ± 0^{a}	0.9 ± 0.1^{a}	5.5 ± 4.7^{ab}	0 ± 0^{a}	0 ± 0^{a}	0.9 ± 0.1^{a}	0 ± 0^{a}	0 ± 0^{a}
Salt	F	0 ± 0^{a}	0.9 ± 0.1^{a}	5.0 ± 4.7^{ab}	0 ± 0^{a}	0 ± 0^{a}	1.1 ± 0.1^{b}	0 ± 0^{a}	0 ± 0^{a}
10 g L^{-1}/144 h	M	0 ± 0^{a}	0.9 ± 0.1^{a}	5.1 ± 4.7^{ab}	0 ± 0^{a}	0 ± 0^{a}	0.9 ± 0.1^{a}	0 ± 0^{a}	0 ± 0^{a}
Form	F	0 ± 0^{a}	0.9 ± 0.1^{a}	5.2 ± 4.7^{ab}	0 ± 0^{a}	0 ± 0^{a}	1.1 ± 0.1^{b}	0 ± 0^{a}	0 ± 0^{a}
0.015 ml L^{-1}/48 h	M	0 ± 0^{a}	0.9 ± 0.1^{a}	3.0 ± 2.5^{a}	0 ± 0^{a}	0 ± 0^{a}	0.9 ± 0.1^{a}	0 ± 0^{a}	0 ± 0^{a}

Ljubobratović et al. (2017) used outdoor ponds for overwintering RAS-reared pikeperch broodstock and found low post-spawning mortality (0–33.3%). These results were similar to those of Zakęś et al. (2013) who used RAS pikeperch broodstock for out of season spawning with mortality in females of 0–16.7% 14 days post-spawning. They did not provide information for male mortality. These data agree with reports that domesticated or RAS-cultured pikeperches have lower stress level and mortality (Łuczyński et al. 2007; Zakęś and Demska-Zakęś 2009; Douxfils et al. 2015). Ljubobratović et al. (2017) found lower post-spawning mortality in males compared with females, which was confirmed by the current study in Phases 1 and 3. In addition to broodstock mortality after nest spawning, Żarski et al. (2013) reported mortality of stripped pikeperch broodstock during spawning at 13 and 15 °C to be 0–33% and 0–50% respectively. Mortality rates fluctuated and were higher compared with broodstock mortality directly after spawning in the present study (2.9–6.7%). These differences could be the result of frequent fish manipulation and handling, necessary for identification of female ovulation, and the greater stress of artificial stripping compared with nest spawning (Blecha et al. 2016a). Rónyai (2007) found higher post-spawning mortality of stripped pond-cultured females (100%) compared with 60% mortality rate of females of the same origin 5 days after nest spawning.

High post-spawning broodstock mortality was identified in this study, as by others (Rónyai 2007; Zakęś and Demska-Zakęś 2009), as a problem related to fish welfare and protection of animals, production costs, and sustainable broodstock management in farms using pond-cultured pikeperch. The chemicals used in this study and their concentrations and duration of exposure were selected and tested according to Gomułka et al. (2007), Svobodová et al. (2007), Mifsud and Rowland (2008), and Kouba et al. (2010). Both salt and formalin are widely used in aquaculture for controlling bacteria, fungi, and parasites in eggs, larvae, juveniles, ongrowing and grow-out stages and broodstock (Svobodová et al. 2007; Kouba et al. 2010; Rodger and Phelps 2015; Leal et al. 2018). Salt is an environmentally friendly and natural agent (Policar et al. 2011c) commonly applied in short (15–30 min) or long baths (24–48 h) at 10–30 g L^{-1} or 1–2 g L^{-1}, respectively (Svobodová et al. 2007). Salt baths to 240 min at 10–15 g L^{-1} and those up to 144 h (6 days) with concentrations at 10 g L^{-1} are shown to be safe and effective for post-spawning treatment of pikeperch broodstock, as the species is physiologically adapted to salinity up to 12–16 g L^{-1} (Brown et al. 2001). Results of Phases 2 and 3 of this study agree with the reported salinity tolerance of pikeperch; salt concentrations 2.5–10 g L^{-1} for 144 h were identified as the most effective in reducing morbidity and mortality of broodstock post-spawning. The salt baths are recommended for post-spawning application to pikeperch broodstock to ensure their survival to the next succeeding spawning seasons. Salt baths are safe for the environment, fish, and farm staff; are low cost; and are easily obtained and applied (Svobodová et al. 2007). Forty-eight hours of formalin exposure at 0.015 ml L^{-1} was equally effective against post-spawning morbidity and mortality of pikeperch broodstock. Formalin is currently considered an effective antifungal, antibacterial, and antiparasitic agent approved for use in aquaculture in the USA and the EU (Celada et al. 2004). However, its application in aquaculture presents a potential hazard to fish, the biological filters of RAS, and the aquatic environment, as well as to farm staff because formalin was identified as a carcinogen (Leal et al. 2018), and its use may not be advisable when other effective options are available. Therefore, this study recommends the use of salt exposure at 2.5–10 g L^{-1} for a 144 h and to reserve formalin treatment for a potential backup treatment for post-spawning reduction of morbidity and mortality in pond-cultured pikeperch broodstock.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement This study was performed and experimental broodstock were handled in accordance with the national and international guidelines for the protection of animal welfare (EU-harmonized Animal Welfare Act of the Czech Republic). The experimental unit is licensed (No. 2293/2015-MZE-17214 and No. 55187/2016-MZE-17214 in project NAZV QK1710310) according to the Czech National Directive (Law against Animal Cruelty, No. 246/1992).

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CHAPTER 5

EFFECTS OF SUB-OPTIMAL WATER TEMPERATURES ON THE FEEDING ACTIVITY OF PIKEPERCH (*SANDER LUCIOPERCA*) WITH REGARDS TO PREDATOR'S SEX AND PREY SIZE

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My share on this work was about 60 %.

EFFECTS OF SUB-OPTIMAL WATER TEMPERATURES ON THE FEEDING ACTIVITY OF PIKEPERCH (*SANDER LUCIOPERCA*) WITH REGARDS TO PREDATOR'S SEX AND PREY SIZE

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ABSTRACT

This study investigated the aspects of adult 3 years old pikeperch *Sander lucioperca* predation to Topmouth gudgeon (*Pseudorasbora parva*) focusing on predator's sex, prey size preference, and the effect of water temperature on feeding activity. The experimental design included three temperature trials: 4.5 C°, 8.5 C° and 12.5 C° of 12 days duration each, and 7 days in between for adaptation and gradual change of photo-thermal conditions. Prey size range included large (PPR = 0.18; predator-prey ratio), medium (PPR = 0.13), and small (PPR = 0.11) fish. The number of consumed prey individuals significantly increased with water temperature rose from 4.5 C° to 8.5 C°. Females showed significantly higher consumption rates throughout the experiment compare to males. The highest relative daily consumption rate was observed at 12.5 °C with females consuming 19.7 ± 6.09 g of prey and males consuming 15.8 ± 4.7 g of prey per one kilo of predator's body weight. Preference for the largest available prey (PPR = 0.18) became significant at water temperatures of 8.5 C° and 12.5 C°. These results could improve understanding of pikeperch winter phenology, predatory behavior and increase its efficiency as an ecological tool for bio-melioration practices in open water bodies.

Keywords: *feeding behavior, trophic ecology, predation, bio-melioration, prey selection, wintering.*

1. Introduction

Pikeperch (*Sander lucioperca*) is a percid with a wide geographic distribution, including the Aral, Azov, Baltic, Black, and Caspian Sea basins, and it occurs in both fresh and brackish waters (Lappalainen et al., 2003; Haponski & Stepien 2013). It is the top predator of many aquatic ecosystems and is valued for its ability to eliminate undesirable fish species from natural water bodies (Keskinen & Marjomäki, 2004; Adamek & Opacak, 2005; Specziár, 2011).

Pikeperch become piscivorous during their first summer (Özyurt et al., 2012; Specziár, 2011). Cyprinids are the most abundant fish in stomach contents of pikeperch (Peltonen et al., 1996). Fish with erectable spines may be avoided, while the shallow bodied species are preferred (Adámek & Opacak, 2005; Pérez-Bote & Roso, 2012). Pikeperch is a gape-limited predator, which leads to the selection of small, as opposed to the largest available, prey (Keskinen & Marjomäki, 2004; Turesson & Brönmark, 2004; Turesson, Persson, & Bronmark, 2002). Topmouth gudgeon (*Pseudorasbora parva*) is one of the most acceptable prey to

pikeperch (Musil & Adámek, 2007). Its invasive abundance and morphological features made it a widely used in aquaculture as a prey species for pikeperch (Malinovskyi et al., 2018; Policar et al., 2016).

Despite the important influence of photo-thermal variations on the biology of pikeperch (Rónyai 2007; Zakęś et al., 2013; Blecha et al., 2015; Ljubobratović et al., 2017), there is little information about winter phenology and the effect of low temperatures and short-days on feeding. Investigations related to winter phenology are important for fisheries management as well as for control of reproduction and general performance of the pikeperch species (Wang et al., 2010; Farmer et al., 2015; Policar et al., 2016; Ljubobratović et al., 2017; Khendek et al., 2018). The vast majority of the studies on pikeperch species have been considering the optimum temperature ranges for the performance of the species while temperatures exceeding those ranges received lack of the scientific attention. This study aimed to determine the effects of sub-optimal water temperatures on feeding activity of pikeperch and the role of predator sex and prey size in this dependence. New findings in predatory behavior might be applied to increase the efficiency of pikeperch as a biomanipulation tool and could contribute to the general understanding of its ecology.

2. Materials and methods

In October 2016, adult pikeperch individuals were collected from the production ponds of the fish farm Nove Hrady Ltd. and transferred to a small earthen pond (0.25 ha) to overwinter at the Faculty of Fisheries and Protection of Waters, University of South Bohemia. In January 2017, experimental fish were transferred to the controlled conditions of a recirculation aquaculture system (RAS). After sex determination using a catheter (Zarski et al., 2012), five males (total length, TL = 473 ± 22 mm and body weight, BW = 1070 ± 100 g) and five females (TL = 464 ± 12 mm and BW = 1060 ± 100 g) were selected for the experiment. Each fish was kept in a separate 350 L tank (10 tanks connected to a RAS) for a 14-day adaptation period at 2.6 °C before the initial trial.

The experiment lasted 64 days from late January through March 2017. Experimental conditions included a gradual increase of water temperature from 4.5 to 8.5, and 12.5 °C for three consecutive thermal trials of 12 days each (Table 1). Each trial was separated by a seven-day rest period during which the temperature was raised. The natural photoperiod was simulated in order to eliminate changes of photo-thermal condition during experimental trials. Artificial light of 50 lux intensity and a duration in accordance with the latitude of Central Europe were applied (Table 1). All changes of photo-thermal regime were performed gradually (0.5 °C and an additional 15 minutes of light duration per day) during adaptation and 7-day rest periods after each experimental trial. Temperature and photoperiod length were consistent over the 12 days of experimental trials.

Each tank was supplied daily with 18 individual prey fish *Pseudorasbora parva* comprising of three size groups within the range of the predator-prey size ratio (PPR) commonly accepted by pikeperch in natural water bodies (Specziár, 2011): six large (TL = 86.6 ± 5.7 mm; BW = 5.76 ± 1.25 g; PPR = 0.18), medium (TL = 63.1 ± 3.5 mm; BW = 2.06 ± 0.4 g; PPR = 0.13), and small (TL = 53.8 ± 3.0 mm; BW = 1.24 ± 0.25 g; PPR = 0.11). No shelter was provided for prey fish. During rest and adaptation periods, all pikeperch were fed ad libitum on the same prey species and size.

Feeding activity of all fish was monitored every 24 hours in the morning during the first hour of the light period. Daily number and size of consumed prey were determined by counting the survived prey fish per each pikeperch female and male. The prey fish were held in the same photo-thermal conditions as the pikeperch. To avoid a learning effect, survived prey was

replaced daily with individuals that had no prior contact with the predators. Relative daily prey consumption rate was calculated as a relation of weight of consumed prey (g) to weight of the predator (kg).

The number of degree days (°d) on a given day of the experiment (n) was counted starting from the day of transportation from pond to RAS (°d = 0) in accordance with the following formula:

$^{\circ}d_n = t_n + d_{n-1}$

Where *n* is a day of experiment, is mean water temperature during the day in the RAS, and $^{\circ}d_{n-1}$ is the value of degree days of the previous day. Daily prey consumption in prey pieces relative to degree days was calculated for each trial (Fig. 1).

After the experiment, all pikeperch were used for semi-controlled reproduction in earthen pond according to Malinovskyi et al. (2018) and spawning success was observed. Twenty four hours after detection of the spawning, nests with deposited eggs were moved to controlled conditions of RAS for the incubation under controlled condition. The hatching rate (%) was investigated from 100 randomly selected live eggs divided equally between two 250 ml plastic jars. After hatching, nests were removed from each tank, and larvae were concentrated in a 10 l volume for counting (Blecha et al., 2016a). For determination of the hatching rate (%), the number of larvae in plastic jars were counted manually (Policar et al., 2011).

All the manipulations with fish during the experiment were done in accordance to the directive 2010/63/EU on the protection of animals used for scientific purposes and performed in accordance with good veterinary practice.

2.1. Statistical analysis

Normal distribution of daily prey consumption data were confirmed by Shapiro-Wilk's tests. After the assumptions for the parametric test were confirmed ($P < .05$), differences in daily prey consumption were estimated using analysis of variance (two-way ANOVA) followed by post-hoc Tukey's test. Differences in prey size selection between males and females at the different temperatures were estimated using the Friedman test. The relationship between degree days and daily prey consumption in pcs was described by a simple linear regression (Fig. 1). All analyses were conducted in Statistica v. 13 (StatSoft, Inc.). Values are presented as Mean ± SD (standard deviation), using a significance level of $P < .05$.

3. Results

Water temperature significantly influenced pikeperch daily prey consumption [$F_{(2, 66)} = 122.60$; $P < .05$]. For both females and males the relative daily prey consumption rate significantly increased with water temperature rose (Fig. 1). In all temperature trials, females showed 24 ± 4.8% [$F_{(1, 66)} = 4.4591$; $P < .05$] higher consumption rate compared to males expressed in both total biomass and number of prey individuals consumed (Table 2).

At 4.5 °C both males and females did not demonstrate significant preference to the prey size, however large and medium individuals were consumed more often. At 8.5 °C significant preference to large and medium prey, rather than the small prey was found in both sexes [$P = .0324$ for male and $P = .0017$ for female; Table 2]. At 12.5 °C males preferred the large prey size [$P = .0057$] while females showed no preference to the prey size.

The mean prey consumption rate did not remain stable during the course of the experiment with peaks occurring over time for each temperature trial. In both sexes peaks of the prey consumption occurred every 2–3 days during the 12.5 °C trial, every six days for females during the 8.5 °C trial and were not observed at 4.5 °C in any of the sexes. (Fig. 1).

Within 8 days post stocking the earthen pond for semi-controlled reproduction all fish spawned successfully. Hatching rate was 75.1 ± 20.7% and larval production accounted 88 700 ± 31 100 per nest (one pair).

4. Discussion

This study investigated the effect of sub-optimal water temperatures on feeding activity of adult pikeperch and preference to the size of the prey. The physiological processes in fish are affected by changes in water temperature and/or photoperiod (Migaud et al., 2010; Wang et al., 2010). Determination of optimal temperature ranges is strategically important from the fisheries management point of view. There is a link between the predator's feeding consumption and the prey availability which are both affected by water temperature (Garvey et al., 1998). Thus, temperature fluctuations will affect individual risk of predator's starvation and subsequent primary (over-wintering survival) and secondary (reproduction outputs of subsequent spring spawning) success of the species (Garvey et al., 1998; Brodersen et al., 2011; Farmer et al., 2015). Pikeperch seem highly plastic to a wide temperature range, with optimal growth between 21–27 °C (Hokanson, 1977; Lappalainen et al., 2003; Hermelink et al., 2011). However, winter phenology of pikeperch receiving lack of scientific attention (Farmer et al., 2015).

Throughout this study female's daily prey consumption was significantly higher than in males. Female energy demand may be higher at late phases of gametogenesis, which may have been the case in this study, resulting in increased daily prey consumption. Özyurt et al. (2012) described seasonality in pikeperch predation, attributing higher consumption in spring to the gamete maturation and reproduction period, although sex was not considered. Saulamo & Lappalainen (2007), investigated the effects of abiotic factors on pikeperch movements, and reported that a relationship exists between increased numbers of pikeperch in captures and rise of water temperature. Guler et al., (2007) found the fatty acid composition of pikeperch muscle to be significantly influenced by spawning and season, probably related to physiological changes during gamete maturation and different provision of fatty acids in the food source. Poulet et al. (2005) reported females to be more active in foraging compared to males and that the foraging activity level of both sexes was related to water temperature, because of increased energy needs. Results of this study are in line with these authors reporting about pikeperch female's higher energy demands.

It may be concluded that the tested feeding regime, and total biomass of the consumed prey during this experiment, was appropriate for the final gamete maturation and spawning. The percentage of hatched larvae was in range of 55-95 % which is in line with reports of other authors that have used pond cultured pikeperch for reproduction (Blecha et al., 2016b; Ljubobratović et al., 2017). Despite the fact that technically fish were fed ad libitum during the course of the experiment, exposure to artificial environment did not affect its reproduction efficiency.

Preference for the larger offered prey fish was significant at 8.5 °C in both sexes as well as at 12.5 °C in males. It may be suggested than preference prey size was related to the predator's unsuccessful attacks resulting in selection of smaller prey. Turesson et al. (2006) reported active selection of the smallest prey fish available and suggested that pikeperch choose small prey to maximize energy intake per unit of time. Studies of prey size selection by pikeperch have shown a positive linear relationship between predator and prey total length (Dörner et al., 2007; Keskinen & Marjomäki, 2004; Specziár, 2011). However, there are no available reports of adult pikeperch prey size preference relative to water temperature. Also, none of the authors have mentioned the effect of temperature on the prey activity, which is a crucial factor of predator's efficiency.

Despite the trend of favoring large prey with rising water temperature, females showed no size preference at 12.5 °C. It is possible that to fulfill increasing energy demands, females consumed all available prey fish to sustain maximum energy intake, while males continued to maintain size preferences. Common PPR for pikeperch in nature water bodies is reported to be near to 0.4 in the one-year-old fish (TL = 200 mm) and 0.26 in larger adult fish (Keskinen & Marjomäki, 2004; Specziár, 2011; Turesson et al., 2006, 2002). In this study, the PPR of large prey was 0.18, and preference for this group may be attributed to its closeness to the optimal size of prey fish for pikeperch (Keskinen & Marjomäki, 2004; Specziár, 2011; Turesson et al., 2006, 2002) or to influance of controlled environment.

The satiation effect may have influenced prey selection and resulted in the periodic peaks of consumption (Fig. 1). Peaks of prey consumption occurred more often in higher temperatures possibly due to faster digestion, and hence pikeperch exhibited the following consumption peak in a shorter period. According to Turesson et al. (2006), digestion of prey by pikeperch takes about 3 days, and daily prey consumption following satiation may influence active prey choice of the predator. Popova & Sytina (1977) reported than pikeperch need 3–4 day to complete digestion in temperature range of 4–18 °C. These results are in line with this study and indicated the effect of water temperature on digestion time.

Although the experiment was conducted in an artificial environment the results provide a general model of pikeperch predation with regards to water temperature, sex of the predator and size of the prey. These findings are useful in terms of fisheries practices and management of fish stocks in water bodies. Data obtained in this study reveals the actual needs of adult pikeperch in prey fish during winter period concluding that when water temperature below 4.5 °C consumption is almost absent. The results of this study are significant in that they show feeding demands of the pikeperch in low temperatures allowing to calculate actual needs in prey fish during winter period. Future research would be required to determine pikeperch predation efficiency in wider temperature ranges. Currently obtained data about the predatory behavior of pikeperch can already be applied to improve feeding practices and provide a higher bio-melioration efficiency in open water bodies and polyculture production ponds.

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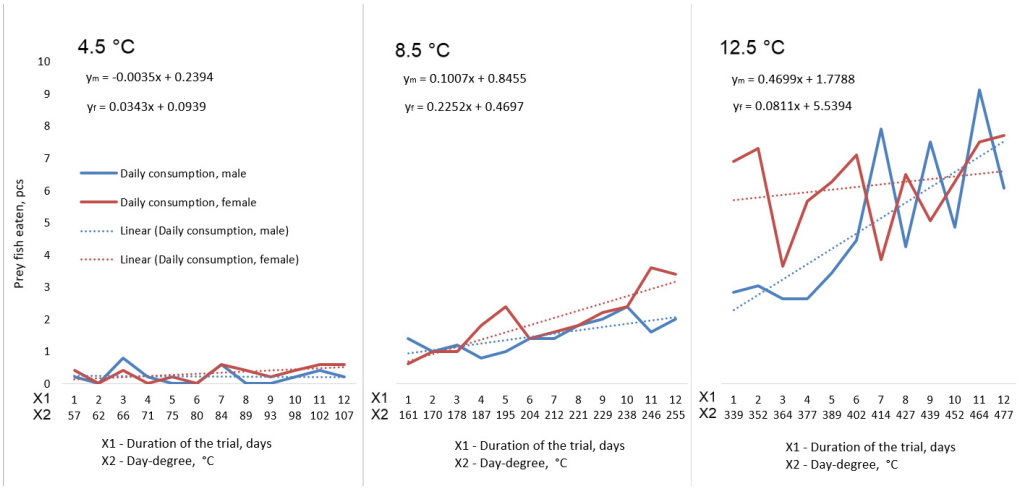

Figure 1. *Mean daily prey consumption of Sander lucioperca males (n=5) and females (n=5) with respect to degree-days during the course of the experiment.*

**y_m - a linear regression equation describing male daily prey consumption*
**y_f - a linear regression equation describing female daily prey consumption*

Table 1*. Experimental set-up and photo-thermal regime during the investigation of feeding behavior of pikeperch (Sander lucioperca).*

Periods	Date	Duration, days	Light duration, h	Temperature, °C
Stocking and adaptation	27.01-09.02	14	8.5	2.6±0.4
First trial	10.02-21.02	12	9.5	4.4±0.2
Rest	22.02-28.02	7	10.25	6.1±1.3
Second trial	01.03-12.03	12	11	8.5±0.2
Rest	13.03-19.03	7	11.75	10.6±1.4
Third trial	20.03-31.03	12	12.5	12.5±0.2

Table 2. *Difference of prey size preference and mean daily prey consumption of pikeperch (Sander lucioperca) relative to water temperature and predator's sex. Daily prey consumption is given as Mean ± SD.*

Temperature [°C]	Sex	Relative daily prey consumption rate, g.kg-1	Mean daily prey consumption, pcs			χ^2 [N = 12, df = 2]	P-value
			Big[1]	Medium[2]	Small[3]		
4.5	♀	1.0±0.3	0.12±0.15	0.12±0.13	0.08±0.13	3.36	p = .1860
	♂	0.6±0.12	0.05±0.12	0.12±0.13	0.05±0.09	0.828	p = .6611
8.5	♀	6.6±2.2	0.82±0.5*	0.68±0.36*	0.43±0.26	12.72	p = .0017
	♂	5.1±1.64	0.62±0.32*	0.55±0.3*	0.33±0.22	6.86	p = .0324
12.5	♀	19.7±6.09	2.35±0.67	1.93±0.63	1.78±0.53	2.26	p = .3229
	♂	15.8±4.7	1.83±0.7*	1.73±0.88	1.32±0.7	10.33	p = .0057

[1] TL = 86.6 ± 5.7 mm
[2] TL = 63.1 ± 3.5 mm
[3] TL = 53.8 ± 3.0 mm

CHAPTER 6

GENERAL DISCUSSION

Investigation of behavioral traits of commercially valuable fish species is strategically important from the aquaculture management point of view (Fontaine, Legendre, Vandeputte, & Fostier, 2009; Khendek et al., 2018; Teletchea, Fostier, et al., 2009). Successful control of reproduction contribute to general performance of the pikeperch species (Teletchea, Gardeur, et al., 2009). Thus, poor organized broodstock management and inappropriate conditions of spawning, egg laying and incubation may cause limitation for successful large-scale pikeperch stocking production (Lehtonen, Lappalainen, Kervinen, & Fontell, 2006). Current pikeperch reproduction protocols are yet to be improved due to the lack of knowledge of their basic ecological and ethological features.

1.1. The substrate selection and spawning behavior

Substrate preferences of pikeperch, as an important part of reproduction biology, and factors influencing its efficiency were investigated in Chapter 2 (Malinovskyi et al., 2018). Pikeperch exhibited preference to thick rigid structure of the brush nest over soft artificial turf fibers, while smooth plastic has not been selected at all. It was the first study of pikeperch selectivity to the type and structure of artificial substrates. Results confirmed opportunistic strategy of pikeperch spawning and emphasized the importance of suitable spawning sites to be available during reproduction period (Lehtonen et al., 2006). Temperature was the only factor affecting spawning occurrence. Fish was tending to select the most suitable type of spawning substrate – brush, even when number of available nests was decreasing. Despite the fact that significant difference in incubation success between two spawning substrate types was not found, there was a tendency to higher larvae production from brush nest compare to artificial turf. In the nature, pikeperch spawn on dense structures such as plant roots and branches (Lappalainen et al., 2003; Saulamo et al., 2005; Lehtonen et al., 2006; Saulamo & Lappalainen, 2007). Gravel or sand is used rarely, and according to current study could indicate lack of the suitable spawning grounds. Investigation of substrate selectivity and the necessary quantity of suitable spawning grounds may significantly improve restocking efficiency, support and sustaining pikeperch populations in nature water bodies (Koed et al., 2000; Lehtonen et al., 2006). Obtained data could be used for indication and supporting of spawning sites in natural water bodies and will contribute to efficient management of wild stock in open water bodies (Lehtonen et al., 2006). Adding of artificial substrate to shallow areas of open waters has been found supportive for several percid species (Nash et al., 1999; Crane & Farrell, 2013). In the same way, spawning opportunities for pikeperch in natural conditions could be increased by providing of artificial spawning substrates.

Artificial spawning nests are also used for semi-controlled reproduction of pikeperch under controlled tank or cage conditions (Steffens et al., 1996; Lappalainen et al., 2003; Zakęś & Demska-Zakęś, 2009). Both natural and synthetic materials (Łuczyński et al., 2007) are used, however, since the structure of the nest is more important than the material of which nest is made (Lehtonen et al. 2006; Čech et al., 2012; Crane & Farrell, 2013), synthetic materials for semi-controlled reproduction are more preferred due to water contamination issues occurred when nest incubated in RAS. Degradation of natural materials may produce a favorable environment for fungal diseases (Zarski et al., 2015) and negatively affect incubation success. In this study synthetic materials supported water conditions without organic contamination along with appropriate egg distribution and provide high hatching rates (48–94%) and subsequent larvae production.

1.2. Behavior and physiological status of the broodstock during reproduction in captivity

Spawning in controlled condition often results in poor physiological status of the broodstock and could negatively affect gamete quality (Sarameh et al., 2012, 2013). Physiological responses of pikeperch broodstock during semi-controlled reproduction were investigated through blood analyzing (Chapter 3; Malinovskyi et al., 2019). Main objective of the study was to evaluate changes in physiological status of the broodstock as a stress response to semi-controlled reproduction protocol where fish were let to spawn on a provided artificial spawning substrate. In this study female exhibited significant decrease in physiological status compare to males. Despite obvious higher sensitivity of females during reproduction (Sarameh et al., 2012) the behavioral feature of pikeperch species (male protective behavior; Lappalainen et al., 2003; Zakęś & Demska-Zakęś, 2009) negatively affected physiological and biochemical parameters of blood in both of the sexes. Video monitoring allowed to detect strong aggressive behavior of male toward to female immediately after spawning completion. In the controlled conditions of the reproduction unit, unlike in the nature, female was not able to leave the spawning site and was receiving numerous attacks from the male leading to injures and decrease of physiological status (Lappalainen et al., 2003; Lehtonen et al., 2006; Rónyai, 2007). This study demonstrate that implementation of video monitoring of broodstock behavior will help to provide adequate welfare conditions of broodstock during or after reproduction. Separation of females after spawning completion significantly reduced injuries and positively affected their physiological status.

1.3. Reduction of post spawning mortality of the pikeperch broodstock

The effectiveness of different antifungal treatments on the mortality of pikeperch broodstock after spawning was compared and evaluated to develop effective protocol of preventing post-spawning broodstock losses (Chapter 5; Policar et al., 2019). Post-spawning losses of pikeperch broodstock is consequence of high sensitivity to manipulation and stress during reproduction, skin lesion and secondary fungal infection (Gomułka et al., 2007; Łuczyński et al., 2007; Rónyai 2007; Zakęś & Demska-Zakęś 2009; Ljubobratović et al., 2017). Long term bath of salt and formaldehyde (such as alternative way to salt bath) were found to be the most effective for mortality prevention. It is more likely that cortisol released in to the bloodstream demonstrates immunosuppressive effect resulting in low effectivity of the immune system (Witeska, 2015). By this, longer application of antifungal compounds were the most effective providing recovery time for fish to restore the physiological status. Without application of special treatments subsequent mortality of broodstock reached 100% in 14 days after spawning. Ljubobratović et al., (2017) reported lower post spawning mortality in RAS cultured pikeperch broodstock if fish were in outdoor condition for overwintering before reproduction. Zakęś et al., (2013) used RAS cultured pikeperch for reproduction broodstock and reported about females' mortality at level of 0 – 16.7% in 14 days after spawning. Rónyai (2007) observed 100% mortality of females and 60% of males in pond-cultured broodstock at 5-th day after spawning. Results of these authors are in line in our study with regards to lower male's mortality after spawning. During semi-controlled reproduction (nest spawning) male's protective behavior and limited space of reproduction makes females targeted leading to various injures and decrease in health status (Zakęś & Demska-Zakęś, 2009). Outputs of this study demonstrate the efficiency of the most often used antifungal compounds and provide useful remarks with regards to methodology of their application.

1.4. Foraging behavior of pikeperch

Together with pike and catfish, pikeperch is a top predator in many aquatic ecosystems (Adamek & Opacak, 2005). Its ability to eliminate less valuable fish species in pond aquaculture is highly appreciated (Musil & Adámek, 2007). Winter phenology of pikeperch concerning predatory behavior is not receiving much of scientific attention even though determination of optimal temperature ranges is strategically important from fisheries management point of view (Shuter et al., 2012; Farmer et al., 2015). The aspects of pikeperch predation with consideration of predator's sex, size of the prey and temperature of water were investigated (Chapter 6; Malinovskyi et al., 2019) in order to clarify some gaps in understanding foraging behavior of adult pikeperch. The study focused on sub-optimal temperature range and reveal the actual temperature when pikeperch begins consuming of the prey. It was found that water temperature close to 4.5 °C is a border temperature for pikeperch as a predator. One of the important findings of the study was the difference in prey consumption between males and females. Despite a well-known seasonality in foraging behavior (Turesson & Brönmark, 2004; Turesson et al., 2006; Guler et al., 2008), higher prey consumption of females was not mentioned in published literature before. Throughout the wide range of temperatures during the course of the experiment females always exhibited higher consumption of the prey compare to male. It was reported that rise of water temperature is associated with intensive movement of the fish in nature water bodies (Koed et al., 2000; Vehanen & Lahti, 2003; Poulet et al., 2005; Aarts & Breukelaar, 2017), however there was no evidence of difference in prey consumption between sexes. The physiological changes occurring during reproduction season are the most obvious reason for difference in foraging behavior between sexes (Guler et al., 2007). Gamete maturation may significantly affect physiological state of pikeperch broodstock (Sarameh et al., 2012, 2013). Considering that experiment was carried out in late winter and early spring higher prey consumption of females could be related to maturation and forthcoming reproduction. Besides the difference in prey consumption between the sexes the preference to the size of the prey varied over the course of the temperatures. After water temperature reached 8.5 °C both males and females demonstrated preference of large and medium over the small sized prey. However, at 12.5 °C only males' exhibited preference to the biggest prey available, although total biomass of consumed prey was still significantly higher for both sexes with water temperature rise. This aspect of predatory behavior was not mentioned in published literature before. It is known that there is a positive linear relationship between total length of the predator and the prey (Keskinen & Marjomäki, 2004; Dörner et al., 2007; Specziár, 2011), however temperature factor wasn't considered. It may be suggested that predator efficiency has been affected with temperature both directly (predator's activity) and indirectly (activity of the prey) or maturation, although new investigations are needed to test that hypothesis. Data obtained from the experiment are significantly expand our understanding of predatory behavior of pikeperch broodstock and results are practically applicable in fisheries management and pond aquaculture.

CONCLUSIONS:

The thesis has tackled important problems in broodstock management of pikeperch during reproduction in a close relation to ecology of the species, discussed and presented valuable solutions applicable to improve the effectiveness of pikeperch aquaculture. The specific conclusions are as follows:

- Pikeperch exhibit opportunistic strategy of spawning emphasizing the importance of suitable spawning sites to be available during reproduction period. Neither water temperature nor availability of spawning places affect substrate preference. Hard and dense rigid structures are recommended for spawning nests during reproduction in captivity.
- Spawning in captivity negatively affects the physiological condition of the pikeperch broodstock. Male aggressive behavior towards the female also negatively affects haematological and biochemical parameters of blood in both sexes. Separation sexes after spawning completion significantly reduces the number of injuries and improves broodstock physiological status. .
- Antifungal bath is an efficient tool to decrease post-spawning mortality of pikeperch broodstock during controlled reproduction. Application of antifungal agents in low concentration and during longer period of time has a significantly higher effectiveness against fungal diseases than short term treatment at higher doses.
- Foraging behavior of pikeperch is significantly affected by changes of water temperature. Females exhibited significantly higher prey consumption rate compare to males. Both sexes tend to consume the biggest prey available. Depending on the water temperature, pond-cultured pikeperch broodstock requires a daily supply of the prey fish in range of 0.6-1.0 $g.kg^{-1}$ (4.5 °C) to 16-20 $g.kg^{-1}$ (12.5°C), during wintering.

The obtained results significantly expand our understanding of pikeperch biology with regard to reproduction in captivity. Despite the fact that aquaculture tends to shift from wild to domesticated fish, broodstock originated from lakes, rivers and ponds are still the main source of highest gamete quality. During the last decade, investigation into reproduction performance of broodstock has significantly contributed to the pikeperch aquaculture establishment (Hermelink et al., 2011; Khendek et al., 2018; Roche et al., 2018; Zakęś, 2007; Zarski et al., 2019). Future research should focus on how domestication alters behavior (e.g. spawning ethology, predatory behavior etc.) in comparison to wild pikeperch.

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ENGLISH SUMMARY

Broodstock management of pikeperch (*Sander lucioperca*) and it's effect on eggs and larvae production

Oleksandr Malinovskyi

Major part of pikeperch market production is provided by catches from nature waterbodies. Domestication processes and development of effective reproductive technics significantly contribute global aquaculture of pikeperch. Adequate and well organized broodstock management is one of the key factors for successful large scale fisheries production. This project was undertaken to improve existing reproduction technics through investigation of important ecological features of pikeperch species.

Spawning behavior and selectivity to different types of spawning substrates were investigated in detail. Pikeperch broodstock exhibited opportunistic spawning strategy with preference to thick and rigid structures for egg laying. Males demonstrated similar pattern in preference of spawning media and selected spawning sites 96.5 ± 45.5 h in advance. Spawning occurred mostly in morning hours (65% of spawning events). Spawning success accounted 97% with duration of 14 days from the moment of initial fish stocking. The experiment confirmed strong selectivity to the spawning site since neither water temperature nor availability of spawning places didn't affected substrate preference of pikeperch broodstock.

Spawning in captivity is leads to decrease in physiological status of the pikeperch broodstock. Significantly higher decrease has been observed in females compare to males. Behavior feature of pikeperch species – male protective behavior, in limited space of the reproduction unit is forwarded to female and negatively affected haematological and biochemical parameters of blood in both of the sexes. Separation of the sexes significantly reduces number injuries and improved physiological status of the broodstock.

Various antifungal treatments were tested to prevent mortality of pikeperch broodstock after reproduction in captivity. Application of antifungal bath during longer period of time had significantly higher effectiveness against fungal diseases. Exposure to salt bath during 144 h in concentration of 2.5, 5 and 10 g L^{-1} and formaldehyde during 72 h in concentration 0.015 ml L^{-1} were found the most effective treatments and significantly decreased mortality after spawning to 8.3–10.5 %. Fish treatment with salt bath are environmentally friendly and recommended for practical use as an effective way to prevent mortality of pond-cultured pikeperch broodstock.

Foraging behavior of pikeperch in relation to sub-optimal temperatures was investigated in detail. Average daily biomass of consumed prey significantly increased in temperature range of 4.5 °C to 12.5 °C. Females exhibited significantly higher prey consumption rate in all temperatures compare to male. The highest prey consumption rate was observed at water temperature of 12.5 C° and accounted 18.58 g and 14.77 g per kilo of the predator body weight for females and males respectively. Pikeperch tended to consume largest available prey after water temperature reached 8.5 °C. At 12.5 °C males sustained size preference while female consumed all available prey sizes.

CZECH SUMMARY

Management generačních ryb candáta obecného (*Sander lucioperca*) a jeho vliv na produkci jiker a larev

Oleksandr Malinovskyi

Tržní produkce candáta obecného je stále založena na odlovech divokých populací ryb, které se vyskytují ve volných vodách. Z tohoto důvodu je kontrolovaný a intenzivní chov candáta obecného včetně vývoje nových reprodukčních technik a využití domestikovaných ryb vysoce žádoucí pro navýšení jeho kontrolované tržní produkce. Správný management s generačními rybami je jeden z klíčových faktorů úspěšné reprodukce a následné produkce tržních ryb. Proto je tato disertační práce zaměřena na optimalizaci chovu a využití generačních ryb candáta obecného k reprodukci. Dále jsme se zabývali etologickými a fyziologickými projevy v průběhu reprodukce, vývoje a optimalizací nových postupů při oplození a inkubaci jiker tohoto druhu.

První část práce detailně popisuje výtěrové chování a vhodný výběr výtěrového substrátu u generačních ryb candáta obecného, které pocházely z rybničního chovu. Candát obecný preferuje tvrdý a pevný výtěrový podklad pro kladení a následnou inkubaci jiker. Samci v rámci první studie po nasazení ryb okupovali jednotlivá výtěrová hnízda po 96,5 ± 45,5 h. Vlastní výtěr probíhal z 65 % v ranních hodinách. V průběhu 14tidenního období se jednotlivé páry postupně vytíraly a úspěšnost výtěru celkově činila 97 %. Tato studie v závislosti na teplotě vody nepotvrdila preferenci výtěrového místa, ale potvrdila silnou preferenci k výběru kartáčového hnízda oproti umělé trávě nebo ploché desce.

Druhá studie této disertační práce popisuje fyziologický stav ryb při poloumělém výtěru v nádržích. Signifikantně vyšší stres byl pozorován u samic oproti samcům po celou dobu výtěru. Samec po výtěru striktně ochraňoval jikry. Stísněná plocha nádrže a stres negativně ovlivnily hematologické a biochemické parametry krve u obojího pohlaví. Hned po výtěru bylo nutné samice z nádrže odlovit, čímž došlo ke zlepšení fyziologického stavu ryb obou pohlaví a zabránilo se četným poranění samic.

Ve třetí části bylo testováno využití různých protiplísňových koupelí s cílem snížit povýtěrovou mortalitu generačních ryb candáta obecného. Aplikace dlouhodobé koupele v kuchyňské soli popřípadě ve formaldehydu statisticky výrazně snížila povýtěrovou mortalitu vytřených generačních ryb. Nejefektivnější dávky používané kuchyňské soli byly 5 a 10 g L^{-1} po dobu 144 h a koncentrace formaldehydu (0,015 ml L^{-1}) při délce expozice 72 h. Tyto koupele významně snížily povrchové zaplísnění ryb a následně povýtěrovou mortalitu generačních ryb candáta obecného u obojího pohlaví na úroveň 8,3–10,5 %. Tyto koupele jsou velice praktické a efektivně snižují mortalitu vytřených generačních ryb. Vedle toho, použití kuchyňské soli je také šetrné k životnímu prostředí.

V poslední studii bylo popsáno potravní chování generačních ryb candáta obecného v závislosti na teplotě vody v předvýtěrovém období. Průměrná biomasa zkonzumovaných ryb se signifikantně zvyšovala s nárůstem teploty ze 4,5 °C na 12,5 °C. Obecně v předvýtěrovém období samice zkonzumují více ryb než samci. Při teplotě 12,5 °C samice zkonzumovaly denně 18,58 g ryb na 1 kg generačního hejna oproti 14,77 g u samců. Také byla zkoumána preferovaná velikost potravních ryb. U samců byla preference identifikována od teploty 8,5 °C, kdy byly preferovány větší potravní ryby. Samice preferenci nevykazovaly a konzumovaly všechny předložené velikosti potravních ryb bez rozdílu.

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- CZ.02.1.01./0.0 /0.0/16_025/0007370 Reproductive and genetic procedures for preserving fish biodiversity and aquaculture (2018-2021).
- 060/2016/Z. Grant Agency of University of South Bohemia.

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- QK1710310 Utilization of new biotechnological approaches under Czech aquaculture with the aim to reach effective, high-quality and environmentally friendly fish production (2017–2021).

- QK1820354 Technical and technological inovation of intensive aquaculture based on new knowledge for future effective and stable fish production (2018–2020).
- QK1810296 The use of alternative components and innovative techniques in fish nutrition (2018–2022)
- QJ1510117 Optimization of methods in artificial and semi-artificial fish reproduction (2015–2018).

LIST OF PUBLICATIONS

Peer reviewed journals

Blecha, M., **Malinovskyi, O**., Veselý, L., Křišťan, J., Policar, T., 2019. Swim bladder inflation failure in pikeperch (*Sander lucioperca*) larvae in pond culture. Aquaculture International 27: 983–989. (IF 2018 = 1.455)

Malinovskyi, O., Kolářová, J., Blecha, M., Stará, A., Velíšek, J., Křišťan, J., Policar, T., 2019. Behavior and physiological status of pond-cultured pikeperch (*Sander lucioperca*) broodstock effected by sexual interactions throughout semi-artificial reproduction. Aquaculture International 27: 1093–1107. (IF 2018 = 1.455)

Policar, T., **Malinovskyi, O**., Kristan, J., Stejskal, V., Samarin, A.M., 2019. Post-spawning bath treatments to reduce morbidity and mortality of pond-cultured pikeperch (*Sander lucioperca* L.) broodstock. Aquaculture International 27: 1065–1078. (IF 2018 = 1.455)

Blecha, M., Dzyuba, B., Boryshpolets, S., Horokhovatskyi, Y., Dadras, H., **Malinovskyi, O.**, Sampels, S., Křišťan, J**.,** Policar, T., 2018. Spermatozoa quality and sperm lipid composition in intensively cultured and wild burbot *Lota lota*. Animal Reproduction Science 198: 129–136. (IF 2018 = 1.817)

Křišťan, J., Zarski, D., Blecha, M., Policar, T., **Malinovskyi, O.**, Samarin, A., M., Palinska-Zarska, K., Nowosad, J., Krejszeff, S., Kucharczyk, D., 2018. Fertilizing ability of gametes at different post-activation times and the sperm-egg-ratio in the artificial reproduction of pikeperch *Sander lucioperca*. Aquaculture Research 49: 1383–1388. (IF 2018 = 1.502)

Malinovskyi, O., Veselý, L., Blecha, M., Křišťan J., Policar T., 2018. The substrate selection and spawning behaviour of pikeperch *Sander lucioperca* L. broodstock under pond conditions. Aquaculture Research: 49: 3541–3547. (IF 2018 = 1.502)

Policar, T., Bondarenko, V., Bezusyj, O., Stejskal, V., Křišťan, J., **Malinovskyi, O.,** Imentai, A., Blecha M., Pylypenko, Y., 2018. Crayfish in central and southern Ukraine with special focus on populations of indigenous crayfish *Astacus pachypus* (Rathke, 1837) and their conservation needs. Aquatic Conservation: Marine and Freshwater Ecosystems 28: 6–16. (IF 2018 = 2.935)

Policar, T., Blecha, M., Křišťan, J., Mraz, J., Velisek, J., Stara, A., Stejskal, V., **Malinovskyi, O.**, Svacina, P., Samarin, A.M., 2016. Comparison of production efficiency and quality of differently cultured pikeperch (*Sander lucioperca* L.) juveniles as a valuable product for ongrowing culture. Aquaculture International 24: 1607–1626. (IF 2016 = 1.095)

Manuscripts

Malinovskyi, O., Blecha, M., Křišťan, J., Policar, T. Effects of the sub-optimal water temperatures on the feeding activity of pikeperch (*Sander lucioperca*) with regards to predator's sex and size of the prey. Aquaculture Research. (Submitted). (IF 2018 = 1.502)

Abstracts and conference proceedings

Malinovskyi, O., Kolářová, J., Blecha, M., Křišťan, J., Stará, A., Velíšek, J., Policar, T., 2018. Hematological and biochemical changes in blood of pikeperch (*Sander lucioperca*) throughout semi-controlled reproduction. In: Sorgeloos P. (ed.): We R Aquaculture, AQUA 2018, USB of Abstracts, Montpellier France, August 25–29, 2018, p. 483.

Policar, T., **Malinovskyi, O.,** Blecha, M., Křišťan, J., Samarin, A.M., 2018. Post-spawning treatment and mortality elimination after two different spawning techniques in pond-cultured pikeperch (*Sander lucioperca*) broodstock. In: Sorgeloos P. (ed.): We R Aquaculture, AQUA 2018, USB of Abstracts, Montpellier France, August 25–29, 2018, p. 603.

Policar, T., Blecha, M., Křišťan, J., **Malinovskyi, O.,** Vaniš, J., 2017. Může být kombinace rybničního a intenzivního chovu candáta obecného (*Sander lucioperca* L.) úspěšně využita v rámci českého produkčního rybářství? In: Urbánek, M. (ed.): 4. ročník odborné konference Rybářské sdružení České republiky. Sborník referátů z odborné konference, České Budějovice 9.–10. února 2017, s. 33–41.

Malinovskyi, O., Křišťan, J., Blecha, M., Veselý, L., Policar, T., 2017. Foraging behavior of pikeperch, *Sander lucioperca* (L., 1758) broodstock. In: Myrseth B. (ed.): Cooperation for Growth, Aquaculture Europe 17, USB of Abstracts, Dubrovnik Crotia, October 17–20, 2017, pp. 706–707.

Křišťan, J., Zarski, D., Blecha, M., Policar, T., **Malinovskyi, O.,** Samarin, A.M., Palinska-Zarska, K., Nowosad, J., Krejszeff, S., Kucharczyck, D., 2018. Fertilizing ability of gametes at different post-activation times and the sperm-egg ratio in the artificial reproduction of pikeperch *Sander lucioperca*. In: Pšenička, M., Fučíková M., Dvořáková, Z. (eds.): 6th International Workshop on the Biology of Fish Gametes, Abstract Book, Vodňany, Czech Republic, September 4–7, 2017, p. 104.

TRAINING AND SUPERVISION PLAN DURING STUDY

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Applied hydrobiology	2017
Ichthyology and fish taxonomy	2016
English language	2018
Biostatistics	2017

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Malinovskyi, O., Kolářová, J., Blecha, M., Křišťan, J., Stará, A., Velíšek, J., Policar, T., 2018. Hematological and biochemical changes in blood of pikeperch (*Sander lucioperca*) throughout semi-controlled reproduction. In: Sorgeloos P. (ed.): We R Aquaculture, AQUA 2018, USB of Abstracts, Montpellier France, August 25–29, 2018, p. 483.	2018
Malinovskyi, O., Křišťan, J., Blecha, M., Veselý, L., Policar, T., 2017. Foraging behavior of pikeperch, *Sander lucioperca* (L., 1758) broodstock. In: Myrseth B. (ed.): Cooperation for Growth, Aquaculture Europe 17, USB of Abstracts, Dubrovnik Crotia, October 17–20, 2017, pp. 706–707.	2017

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Università Degli Studi di Parma, Italy. Supervisor: prof. Francesco Nonnis Marzano. Reproduction and broodstock management of endemic species of Mediterranean barbel (*Barbus meridionalis*) (4 weeks)	2016
Inagro vzw, Belgium. Supervisor: Dr. Stefan Teerlinck. Out-of-seasonal artificial reproduction of pikeperch. Broodstock management, incubation and first feeding of larvae (4 weeks)	2017
Research Institute for Fisheries and Aquaculture NAIK HAKI, Hungary. Supervisor: Dr. Uros Ljubobratović. Out-of-seasonal artificial reproduction of pikeperch. Broodstock management, incubation and first feeding of larvae (2 weeks)	2018
University of Warmia and Mazury in Olsztyn, Department of Lake & River Fisheries Supervisor: prof. dr hab. inż. Dariusz Kucharczyk. Optimization of intensive culture in larvae and juveniles of pikeperch, burbot and European catfish	2019

Pedagogical activities	**Year**
Leading of project entitled Foragying behaviour of pikeperch at Summer school	2018
Anouncing the project entitled Interspecific competition in pikeperch at Summer school	2019
Lecturing of students of bachelor study, discipline Fishery at USB FFPW in range of 48 teaching hours	2016–2019
Consultation of students during thesis preparation, statistical analyzing of the data in range of 44 teaching hours	2016–2019

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