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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

Brook trout (*Salvelinus fontinalis* Mitchill)

Brook trout is one of the representatives of the Salmonidae family and the Salmoninae subfamily. In addition, this family is represented by salmon, trout, charr, whitefish, and cisco (Karas, 2015). As a species, brook trout is technically considered charr, or as a member of the genus *Salvelinus*. It is assumed that this species, along with the lake trout, *Salvelinus namaycush*, is the main member of the genus from which other species have arisen (Crespi and Fulton, 2004). In one of the published works of Hoar (1976), it was suggested that the genus *Salvelinus* has the most pronounced similarities with the earliest salmoninae – a 'primitive archetype' from which *Salmo* and *Oncorhynchus* were further specialized. Within the Salmonidae taxonomic family, the genus *Oncorhynchus* is the most closely related to *Salvelinus*, and the fish in these two genera share many similar basic characteristics. However, the relationship to other species within the genus is still yet to be fully explored, presumably due to extensive hybridization (Crespi and Fulton, 2004). Currently, it has been documented that in hatcheries, brook trout have been crossed with brown trout (*Salmo trutta*) to produce tiger trout (*S. fontinalis × S. trutta*), with Arctic charr (*Salvelinus alpinus*) to produce sparctic trout (*S. fontinalis × S. alpinus*), or with lake trout (*Salvelinus namaycush*) to produce splake (*S. fontinalis × S. namaycush*), and it has also been hybridized with bull trout (*Salvelinus confluentus*) (Dupont Cyr et al., 2018; Feringa et al., 2016; Hansen, 2019).

Brook trout has a pronounced distinctive phenotypic feature and its distinguishing feature is the presence of yellow vermiculations on the dorsal surface and dorsal fins. Other marking useful in identifying brook trout is the combination of pale-yellow spots and red spots encircled by a light blue halo (Behnke, 2002). The fins on the ventral surface are bordered by white markings and can vary in color from yellow to a vivid red — a feature common to many members of the genus *Salvelinus* (Behnke, 2002). The pelvic and anal fins are usually dark orange in color and have black pigment behind a white leading edge. The caudal fin is blunt or slightly forked. One of the methods for identifying a sexually mature male among brook trout is the presence of a dark orange and black colouration on their abdominal area (Ficke et al., 2009).

Three main life history forms of brook trout can be distinguished: stream resident, lacustrine, and anadromous (Bridges, 1958). Along with other representatives of charrs, brook trout prefer cool, clear, well-oxygenated waters. But it should also be noted that brook trout is the most adapted to warmth and thermally tolerant charr species. The preferred average temperature is between 16–20 °C (Behnke, 2002; Coutant, 1977; De Staso and Rahel, 1994).

The largest angler-caught brook trout was a 6.57 kg and approx. 86 cm-long fish caught in 1915 from the Nipigon River, a tributary of Lake Superior (Hansen, 2019).

General habitat and naturalized distribution

Brook trout is known as one of the most widespread and well-adaptive species among *Salvelinus* (Raleigh, 1982). This species is endemic to North America and its natural distribution has been determined from the Atlantic provinces south to Long Island, N. Y.; in the Appalachian Mountains south to Georgia; west in the upper Mississippi and Great Lakes drainages to Minnesota; and north to Hudson Bay (Naiman et al., 1987). Later, this fish species was gradually introduced into the waters and intensive aquaculture systems of North America and Eurasia, Central and South America (Meyer et al., 2006).

The natural habitat of the brook trout is streams and rivers, but it can also be found in the shallower areas of cold lakes (Henderson, 1963). Throughout most of their natural habitat, they enter salt water as much as possible (where acceleration of the growth rate can be

noted). Brook trout has well-developed homing and migration abilities. In the sea, they usually stay for no more than 4–5 months near their natal river (Naiman et al., 1987).

In the Czech Republic, the literature indicates 1885 as the year of the first stocking of brook trout in the Black Lake of the Sumava Mountains in southwestern Bohemia (Kouřil et al., 2008). Naturalized populations have developed in the High Tatra Mountains of northern Slovakia, in middle and eastern Slovakia, in the Hruby-Jesenik Mountains of northern Moravia, in the Bohemian-Moravian Highlands, and in the Krkonose and Jizerske mountains of northern Bohemia (MacCrimmon and Campbell, 1969).

The existing discontinuity in distribution should not be seen as a lack of effort to more widely establish the species, but as an aspect that reflects the sensitivity of the species to environmental conditions, regardless of whether we are considering it on a local, continental or revolt level. In certain locations the introduction of brook trout was so successful that this now threatens the species native to the watersheds, and attempts have been made to eradicate them (Meyer et al., 2006).

Reproduction of brook trout

Reproduction is a long-lasting and very energy intensive physiological process. Generally, it requires the redirection of resources and their relocation from somatic growth. Therefore, the spawning window is highly dependent on the need to optimize the distribution of available energy. This is all achieved through an exact coordination of gonadal recruitment and development under the influence of environmental and ecological signals (Migaud et al., 2010).

Brook trout is a fall-spawning fish species. Spawning usually takes place between August and December when water temperatures begin dropping after high summer temperatures (Behnke, 2002). Sexual maturation of brook trout occurs at the age of 1 to 5 years, and most fish mature by age 3 (Wydoski and Whitney, 2003).

In their natural habitat, brook trout begin the pre-spawning migration process upstream at the end of summer—in search of gravel-bottomed areas in cold tributaries (Heft, 2006). The reproductive activities of females include the creation of a breeding site – redd (nest), serial excavations of stream substrate into which all fertilized egg batches are released, and the subsequent replacement of stream gravel over the egg for protection. Fertilization can be carried out by more than one male. Male reproductive activities include competing for females. This is then followed by eggs fertilization, which can also be from more than one female (Hutchings, 1994). The female remains at the breeding site for a short time after the eggs have been fertilized, and the male leaves this place during this period (Heft, 2006). Fry appear after approximately 140 days at a temperature of 2 °C (Wydoski and Whitney, 2003).

Under laboratory or hatchery conditions of brook trout breeding, size is a more important determinant of maturation. Thus, 97–99 percent of the variation in maturation can be explained by the advantage of the size indicator over the age or growth rate (McCormick and Naiman, 1984). Timing of sexual maturation can normally vary depending on sex. There is also a trend that the proportion of mature males at small sizes is greater than that of mature females (Carlson and Hale, 1973; Power, 1980).

Brook trout production and market

Currently, aquaculture is progressing rapidly and becoming more important. This is due to overexploitation of natural fisheries. Aquaculture has set itself the paramount task of supplying many artificial and natural recreational fisheries, as well as meeting a lot of the commercial and retail demands that continue to grow worldwide (Naylor et al., 2000).

One of the developing areas of aquaculture is brook trout farming. Brook trout have some favorable characteristics for improvement and aquaculture. This species is becoming popular in Central and North Europe because of its high palatability and suitability to rearing in various types of aquaculture systems (Lundova et al., 2019). Also, a positive feature for brook trout aquaculture is the ability to tolerate a wide variety of environmental conditions, including lower water temperatures and pH level than other salmonids. Furthermore, they seem to have some population-dependent differences in their life history, which suggest substantial opportunities for selective breeding (Naiman et al., 1987). Brook trout is known as a valuable sport fish. For this reason, the fish was introduced for recreational fishing around the world. Thus, there are 49 countries where brook trout have become established, making this species the second most widely introduced salmonid fish after rainbow trout *Oncorhynchus mykiss* (Fausch, 2008). However, FAO's (2019) statistics over the past years show, the amount of brook trout produced in aquaculture is significantly lower than Arctic charr and rainbow trout (Figure 1).

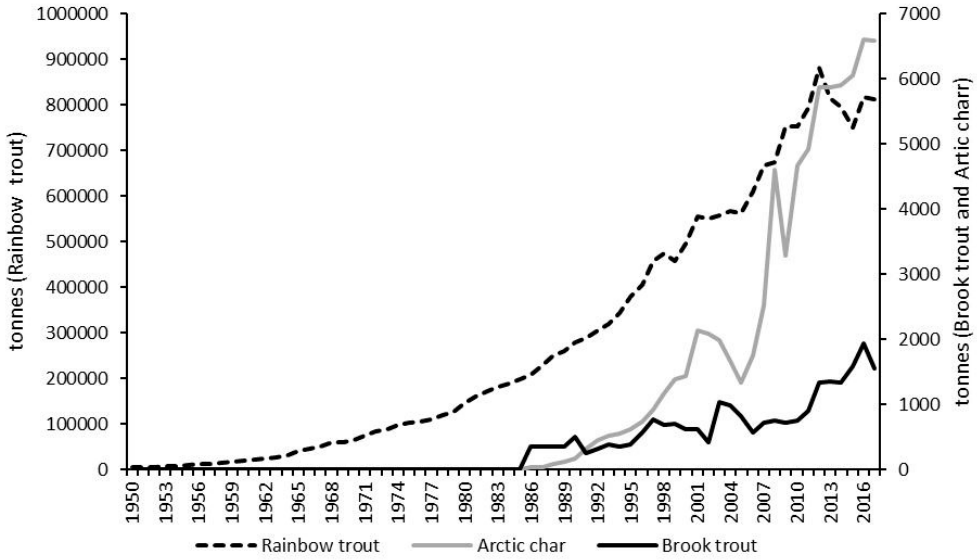

Figure 1. *Global aquaculture production of selected salmonids species for the period 1950–2017.*

It is important to note that the targeted culture of brook trout is relatively new, and attempts to breed these fish have been relatively inconsistent, and studies under commercial conditions have been minimal (Başçinar and Okumuş, 2004).

Photoperiod as a regulatory factor of reproduction cycle in fish

It is a well-known fact that unwanted sexual maturation affects numerous farmed fish species worldwide (Liu and Duston, 2016). The maturing process in teleosts fish is one of the most energy-intensive processes, and involves significant changes in the reproductive structure, metabolism, behavior and fish growth (Ginés et al., 2003; Lundqvist, 1980). It is assumed that due to the interaction between sex hormones and growth hormones, the accumulation of sex steroids often acts as a limiting growth factor (Ginés et al., 2003). When the development of the gonads is stopped, the energy that has been assigned to gonad

development may be redirected to muscle development and the accumulation of fat in the abdominal cavity (Noori et al., 2015). Consequently, delayed puberty is a desirable aspect in many different fish farming programs (Kronert et al., 1989; Longalong et al., 1999; Noori et al., 2015)

Among the methods and approaches for avoiding the negative effects of sexual maturity in fish, the most widely used are hormonal sex reversal, triploid production, and special selection against early maturation (Kause et al., 2003; Longalong et al., 1999; Lorenzen, 2000). Along with the above techniques, it should be noted that the manipulation of environmental factors, such as temperature and photoperiod, also proved to be an effective tool for regulating physiological processes in fish farming (Imsland et al., 2014). According to the previous studies, it can be concluded that the maturation of cyprinids, catfish and other tropical and sub-tropical fish species is influenced by temperature (Davies et al., 1986; Munro, 1990; Peter and Yu, 1997; Sundararaj and Vasal, 1978). Also, in several experiments, it was revealed that for some cyprinids, moronids and percids species, environmental aspects such as photoperiod and temperature are of great importance (Bromage et al., 2001; Clark et al., 2005; Migaud et al., 2002; Prat et al., 1999). However, in this case, it can be assumed that temperature plays a minor role compared to photoperiod, as suggested by the contradictory results obtained in Eurasian perch (Abdulfatah et al., 2007). In turn, based on a range of studies, it was suggested that photoperiod is a key environmental factor for the initiation and completion of puberty in fish, ensuring the appropriate seasonal timing of reproduction in accordance with favourable conditions for the offspring (Oppedal et al., 2006; Taranger et al., 2010; Taylor et al., 2008).

Based on numerous previous studies, it has been suggested that the influence of the photoperiod acts through entrainment of circannual endogenous rhythms that control a "gating" or "critical time window" mechanism. This means the period during which puberty is allowed to commence or continue depending on the physiological conditions of the animal, e.g. body size and stage of gonadal development, or being postponed due to the inability of the organism to exceed genetically determined developmental thresholds (Bromage et al., 2001; Oppedal et al., 2006; Taranger et al., 1999; Thorpe, 2004; Thorpe, 2007).

First unambiguous evidences on the influence of daylength on the reproduction cycle in the brook trout (*Salvelinus fontinalis*) were the works of Hoover (1937) and Hoover and Hubbard (1937). These researchers showed that compressing seasonal light cycles into time periods shorter than one year causes to an earlier spawning of 3–4 months—in comparison with the spawning under ambient conditions (Bromage et al., 2001). Successively, Hazard and Eddy (1951), Corson (1955) and Carlson and Hale (1973) carried out further studies on this issue regarding the effects of seasonal photoperiods on the spawning time of brook trout.

Over the past few decades, photoperiod techniques have been found to be a positive influence on fish growth, reproduction and gonadal development. In this regard, these techniques are currently widely used in aquaculture to regulate spawning and maturation cycles, and stimulate fish growth (Biswas and Takeuchi, 2002; Bromage et al., 2001; Ginés et al., 2003; Noori et al., 2015; Purchase et al., 2000; Randall et al., 2001; Rodrıguez et al., 2001).

Physiological side of photoperiod manipulation

Fish have two main photoreceptive organs: the eyes and the pineal complex, which consists of the pineal gland and parapineal glands (Ekstrom and Meissl, 1997; Falcón et al., 2007). After their transformation into messages, photic signals are transmitted by the retina and the pineal gland to brain centres through a specific population of ganglion cells. The pineal gland is more rudimentary in comparison with the retina as it contains only cone photoreceptor cells that directly contact ganglion cells. The pineal gland acts as a luminance and day-length

detector with rudimentary spectral differentiation. These characteristics are confirmed by studies of tissue culture of the whole gland, as well as electrophysiological recordings from the photoreceptor and ganglion cells (Ekstrom and Meissl, 1997; Vera et al., 2010). The retina's organization is more complex. It consists of a complex vertical and horizontal network of bipolar, amacrine and horizontal cells at the interface between the photoreceptor cells, which in turn comprise cones, rods and the ganglion cells (Migaud et al., 2010).

In vertebrates, the brain-pituitary system is the main element in the control of reproductive processes (including puberty), and fish are no exception (Okuzawa, 2002). It is assumed that the pineal organ and/or the lateral eyes transmit light-induced modulation of the pubertal activation of this system to neuroendocrine neurons, producing gonadotropin-releasing hormone (GnRH). This is followed by changes in testicular physiology provoked by changes in the pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) release, which in parallel leads to a change in the activities of Sertoli and Leydig cells (Schulz et al., 2006). The hypothalamic-pituitary-gonadal axis plays one of the main roles in vertebrate reproduction. The hypothalamus of the brain secretes GnRH, which additionally stimulates the pituitary gland to release two types of gonadotropins (GTHs), namely the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) (Fang, 2018). FSH stimulates ovarian follicles to secrete estradiol-17β (E2), which is responsible for stimulating the liver to produce vitellogenin for the initiation of sexual maturation. LH is responsible for the final stage of maturation (Choi et al., 2010).

It is generally accepted that seasonal changes in photoperiod act as a synchronizer of endogenous rhythms. The photoperiod effect is transformed via the light-brain-pituitary axis involving several endocrine factors such as cortisol, tryroid and growth hormone (Björnsson et al., 2011; Imsland et al., 2014). The teleost pineal gland captures the endogenous rhythmicity of various biological systems through the photo-neuroendocrine transduction of the daily and seasonal photoperiod signal into diel rhythm of hormonal and neural information (De Vlaming and Olcese, 1981; Ekstrom and Meissl, 1997; Mayer et al., 1997; Porter et al., 2001). This process is carried out via melatonin (N-acetyl-5-methoxytryptamine), an indole that is synthesized by pineal photoreceptor cells (Falcón et al., 1992; Falcon et al., 1986). Therefore, melatonin is often called a biological time-keeping hormone, which entrains circadian (a cycle of 24 hour) and circannual (a cycle of 1 year) rhythms (Bromage et al., 2001; Falcón et al., 2007). Nowadays, in accordance with the alleged information that melatonin may not control the initiation of reproduction, it undoubtedly plays an important role in co-ordinating the reproductive development through oocyte maturation and feedback control of gonadotropin signaling (Migaud et al., 2010; Sébert et al., 2008).

To summarize, to continue reproduction, sexual maturation must be attained by activating numerous germ cells and implementing predetermined energic thresholds in an appropriate period of time (which is determined by the prevailing photoperiod). It has been precisely established that the seasonally changing photoperiod, which is the clearest environmental cue, is used to initiate and co-ordinate gonadal development by most temperate fish species (Pankhurst and Porter, 2003).

Photoperiod manipulations in Salmonids aquaculture

Salmonids is a fish family that shows a pronounced dependence on photoperiod for entrainment of their reproduction cycles. It is noteworthy that in the absence of other variable factors, photoperiod can regulate complete reproductive development (Pankhurst and King, 2010). The most common reactions of Salmonids to photoperiod manipulations are changes in the incidence of puberty, and a change in the timing of spawning. The last-mentioned

reaction is a recognized technique to produce gametes throughout the year (Duston et al., 2003). Sexual maturation among salmonids is defined as a threshold trait which proceeds only when an individual exceeds a "threshold". The exact definition of this moment is still not entirely clear, but it is known that it includes important factors such as body size, lipid reserves, growth rate and year season (Liu and Duston, 2016; Sloat et al., 2014). Maturation begins about one year prior to spawning, while maintaining optimal conditions. This process can be registered by the presence of gametogenesis, sexual dimorphic body, increasing the level of fat and the sex steroids level (Andersson et al., 2013; Campbell et al., 2006).

Photoperiod and its manipulations are widely used methods in salmon aquaculture to suppress early sexual maturity. Fish farming is forced to resort to these techniques because of the negative impact of early maturation on growth rate and feed conversion ratio, which also leads to a deterioration of the fish muscle (Good et al., 2015). Seasonal changes in day length regime are thought to be the main environmental aspect of sexual maturation in most fish species (Bromage et al., 2001). Thus, based on scientific studies that have shown an artificially extended photoperiod has some positive effect relative to the natural photoperiod, various artificial photoperiod regimes are used in salmon aquaculture to prevent early maturation (Good et al., 2015; Guerrero-Tortolero and Bromage, 2008; Lundova et al., 2019; Unwin et al., 2005). As a concrete example, one can nowadays cite the widespread use of photoperiod manipulations to reduce the incidence of maturation in rainbow trout (*Oncorhynchus mykiss*) and to reduce the incidence in Atlantic salmon (*Salmo salar*) (Bromage et al., 2001; Duston et al., 2003). However, it should also be noted that some studies showed opposite results, in which an extended photoperiod accelerated fish maturation (Fang, 2018; Imsland et al., 2014). In addition to regulating the reproductive cycle, photoperiod manipulation is also often used to stimulate growth rate in fish farming. The earlier study of Stefansson et al. (1991) may be evidence of this. They determined that freshwater Atlantic salmon parr-smolts had about 15% more growth over 5 months under continuous photoperiod regime, compared with fish reared under natural ambient light regime (Fang, 2018). Similar results of enhanced growth were also obtained by studying the effect of a prolonged photoperiod on rainbow trout (Noori et al., 2015).

First of all, available knowledge about the effect of photoperiod on fish reproduction was used for manipulation of spawning time in aquaculture. The ability to control the spawning process allows the supply of fish material (eggs and/or fry) to fish farms for further rearing during the year, regardless of the normal spawning season of the fish in question (Randall et al., 1995). By using such strategies, fish farming is able to meet the market requirements for year-round production of market-size fish. This is why the question of developing special methodologies to both advance and delay reproduction cycles is becoming increasingly relevant in salmonid aquaculture (Bromage et al., 2001).

Objectives of the Ph.D. thesis

This Ph.D. thesis is focused on an investigation of the effect of a prolonged photoperiod on the sexual maturation, growth rate and overall performance of brook trout. The issue can be divided into the following objectives:

1. Comparison of the effect of various light sources on puberty and growth performance in brook trout.
2. Clarification of the effect of light sources and prolonged photoperiod upon colouration of brook trout.
3. Evaluate the effect of prolonged photoperiod regimes and light sources on the resistance of fish to secondary fungal diseases during a stressful situation.

4. Determination of the most effective timing of the artificially extended photoperiod in order to delay sexual maturation and improve the growth rate of brook trout.
5. Investigate the effect of various non-circadian photoperiod regimes on the growth and puberty of brook trout.
6. Evaluation of the effect of prolonged photoperiod on post-mortem changes, sensory conditions, textural and nutritional characteristics of brook trout flesh.

References

Abdulfatah, A., Fontaine, P., Marie, M., 2007. Effects of the photoperiodic and thermal kinetics on the induction of the reproduction cycle in the perch (*Perca fluviatilis*). In: Abstract Book of the Eigth International Symposium on Reproductive Physiology of Fish, Saint-Malo, France, 3–8 June 2007, p. 203.

Andersson, E., Schulz, R.W., Male, R., Bogerd, J., Patiña, D., Benedet, S., Norberg, B., Taranger, G.L., 2013. Pituitary gonadotropin and ovarian gonadotropin receptor transcript levels: seasonal and photoperiod-induced changes in the reproductive physiology of female Atlantic salmon (*Salmo salar*). General and Comparative Endocrinology 191, 247–258.

Başçinar, N., Okumuş İ., 2004. The early development of brook trout *Salvelinus fontinalis* (mitchill): Survival and growth rates of alevins. Turkish Journal of Veterinary and Animal Sciences 28, 297–301.

Behnke, R.J., 2002. Trout and Salmon of North America. The Free Press, Simon and Schuster, Inc., New York, 363 pp.

Biswas, A.K., Takeuchi, T., 2002. Effect of different photoperiod cycles on metabolic rate and energy loss of fed and unfed adult tilapia *Oreochromis niloticus*: Part II. Fisheries science 68, 543553.

Björnsson, B.T., Stefansson, S.O., McCormick, S.D., 2011. Environmental endocrinology of salmon smoltification. General and Comparative Endocrinology 170, 290–298.

Bridges, C.H., 1958. A compendium of the life history and ecology of the Eastern Brook Trout (*Salveninus fontinalis* Mitchell). Massachusetts Division of Fish and Game Fisheries Bulletin, 37.

Bromage, N., Porter M., Randall C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197, 63–98.

Campbell, B., Dickey, J., Beckman, B., Young, G., Pierce, A., Fukada, H., Swanson, P., 2006. Previtellogenic oocyte growth in salmon: relationships among body growth, plasma insulin-like growth factor-1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic-acute regulatory protein and receptors for gonadotropins, growth hormone, and somatolactin. Biology of Reproduction 75, 34–44.

Carlson, A.R., Hale, J.G., 1973. Early maturation of brook trout in the laboratory. The Progressive Fish-Culturist 35, 150–153.

Choi, S., Lee, C.H., Park, W., Kim, D.-J., Sohn, Y.C., 2010. Effects of shortened photoperiod on gonadotropin-releasing hormone, gonadotropin, and vitellogenin gene expression associated with ovarian maturation in rainbow trout. Zoological Science 27, 24–32.

Clark, R.W., Henderson-Arzapalo, A., Sullivan, C.V., 2005. Disparate effects of constant and annually-cycling daylength and water temperature on reproductive maturation of striped bass (*Morone saxatilis*). Aquaculture 249, 497–513.

Corson, B., 1955. Four years' progress in the use of artificially controlled light to induce early spawning of brook trout. The Progressive Fish-Culturist 17, 99–102.

Coutant, C.C., 1977. Compilation of temperature preference data. Journal of the Fisheries Research Board of Canada 34, 739–745.

Crespi, B.J., Fulton, M.J., 2004. Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny. Molecular Phylogenetics and Evolution 31, 658–679.

Davies, P.R., Hanyu, I., Furukawa, K., Nomura, M., 1986. Effect of temperature and photoperiod on sexual maturation and spawning of the common carp: II. Under conditions of low temperature. Aquaculture 52, 51–58.

De Staso, J., Rahel, F.J., 1994. Influence of water temperature on interactions between juvenile Colorado river cutthroat trout and brook trout in a laboratory stream. Transactions of the American Fisheries Society 123, 289–297.

De Vlaming, V., Olcese, J., 1981. The pineal and reproduction in fish, amphibians and reptiles. in: Reiter, R. (Ed.), The pineal gland, Vol. 2, Reprosuctive effects. Boca Raton: CRC Press, pp. 2–21.

Dupont Cyr, B.-A., Dufresne, F., Christen, F., Desrosiers, V., Proulx, É., Le François, N.R., Vandenberg, G.W., Blier, P.U., 2018. Hybridization between char species (*Salvelinus alpinus* and *Salvelinus fontinalis*): a fast track for novel allometric trajectories. Biology Open 7, bio033332.

Duston, J., Astatkie, T., MacIsaac, P., 2003. Long-to-short photoperiod in winter halves the incidence of sexual maturity among Arctic charr. Aquaculture 221, 567–580.

Ekstrom, P., Meissl, H., 1997. The pineal organ of teleost fishes. Reviews in Fish Biology and Fisheries 7, 199–284.

Falcón, J., Bégay, V., Besse, C., Ravault, J.-P., Collin, J.-P., 1992. Pineal photoreceptor cells in culture: fine structure, and light/dark control of cyclic nucleotide levels and melatonin secretion. Journal of Neuroendocrinology 4, 641–651.

Falcón, J., Besseau, L., Sauzet, S., Boeuf, G., 2007. Melatonin effects on the hypothalamo-pituitary axis in fish. Trends in Endocrinology & Metabolism 18, 81–88.

Falcon, J., Voisin, P., Guerlotte, J., Collin, J., 1986. Photoreceptors in the teleost pineal organ. Daily fluctuations of indole metabolism, Annales d'endocrinologie, pp. 65–66.

Fang, Y., 2018. Effects of salinity and photoperiod on growth, aerobic scope, and hypoxia tolerance of Atlantic and coho salmon in recirculating aquaculture systems. University of British Columbia, Vancouver, pp. 102.

FAO, 2019. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2017 (FishstatJ). Retrieved from http://www.fao.org/fishery/statistics/software/fishstatj/en.

Fausch, K.D., 2008. A paradox of trout invasions in North America. Biological Invasions 10, 685–701.

Feringa, M., Huckins, C., Mattes, W., Baker, E., Zorn, T., Littlefield, J., Scribner, K., 2016. Genetic and phenotypic evidence for splake presence in brook trout and lake trout spawning habitats. Journal of Great Lakes Research 42, 738–742.

Ficke, A., Peterson, D., Janowsky, B., 2009. Brook trout (*Salvelinus fontinalis*): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region.

Ginés, R., Afonso, J., Argüello, A., Zamorano, M., Lopez, J., 2003. Growth in adult gilthead sea bream (*Sparus aurata* L) as a result of interference in sexual maturation by different photoperiod regimes. Aquaculture Research 34, 73–83.

Good, C., Weber, G.M., May, T., Davidson, J., Summerfelt, S., 2015. Reduced photoperiod (18 h light vs. 24 h light) during first-year rearing associated with increased early male maturation in Atlantic salmon (*Salmo salar)* cultured in a freshwater recirculation aquaculture system. Aquaculture Research 47, 3023–3027.

Guerrero-Tortolero, D.A., Bromage N., 2008. Growth and maturation of Atlantic salmon (*Salmo salar*) populations with different grilse proportions under natural photoperiod and superimposed nighttime light. Aquaculture 285, 63–66.

Hansen, M.J.G., Guy, Ch.S., Budy, P., McMahon, T.E., 2019. Trout as native and non-native species: A management paradox. Trout and Char of the World, 645–684.

Hazard, T.P., Eddy, R.E., 1951. Modification of the sexual cycle in brook trout (*Salvelinus fontinalis*) by control of light. Transactions of the American Fisheries Society 80, 158–162.

Heft, A.A., 2006. Maryland Brook Trout Fisheries Management Plan. Maryland Department of Natural Resources Fisheries Service Inland Fisheries Management Division DCNR Bureau of Forestry, 130 pp.

Henderson, N.E., 1963. Influence of light and temperature on the reproductive cycle of the Eastern brook trout, *Salvelinus fontinalis* (Mitchill). Journal of the Fisheries Research Board of Canada 20, 859–897.

Hoar, W.S., 1976. Smolt Transformation: Evolution, Behavior, and Physiology. Journal of the Fisheries Research Board of Canada 33, 1233–1252.

Hoover, E.E., 1937. Experimental modification of the sexual cycle in trout by control of light. Science 86, 425–426.

Hoover, E.E., Hubbard H.E., 1937. Modification of the sexual cycle in trout by control of light. Copeia 1937, 206–210.

Hutchings, J.A., 1994. Age- and size-specific costs of reproduction within populations of brook trout, Oikos 70, 12–20.

Imsland, A.K., Handeland, S.O., Stefansson, S.O., 2014. Photoperiod and temperature effects on growth and maturation of pre-and post-smolt Atlantic salmon. Aquaculture International 22, 1331–1345.

Karas, N., 2015. Brook Trout: A Thorough Look at North America's Great Native Trout-Its History, Biology, and Angling Possibilities. Simon and Schuster, pp. 492.

Kause, A., Ritola, O., Paananen, T., Mäntysaari, E., Eskelinen, U., 2003. Selection against early maturity in large rainbow trout *Oncorhynchus mykiss*: the quantitative genetics of sexual dimorphism and genotype-by-environment interactions. Aquaculture 228, 53–68.

Kouřil, J., Mareš, J., Adámek, Z., Randák, T., Kolářová, J., Palíková, M., 2008. Chov lososovitých druhů ryb, lipana a síhů. RIFCH USB, Vodňany, 142 pp. (in Czech)

Kronert, U., Horstgen-Schwark, G., Langholz, H.J., 1989. Prospects of selecting for late maturity in tilapia (*Oreochromis niloticus*): I. Family studies under laboratory conditions. Aquaculture 77, 113–121.

Liu, Q., Duston, J., 2016. Preventing sexual maturation in Arctic charr by 24 h light overwinter and suppressing somatic growth. Aquaculture 464, 537–544.

Longalong, F.M., Eknath, A.E., Bentsen, H.B., 1999. Response to bi-directional selection for frequency of early maturing females in Nile tilapia (*Oreochromis niloticus*). Aquaculture 178, 13–25.

Lorenzen, K., 2000. Population dynamics and management, Tilapias: biology and exploitation. Springer, pp. 163–225.

Lundova, K., Matousek, J., Prokesova, M., Vanina, T., Sebesta, R., Urban, J., Stejskal, V., 2019. The effects of a prolonged photoperiod and light source on growth, sexual maturation, fin condition, and vulnerability to fungal disease in brook trout *Salvelinus fontinalis*. Aquaculture Research 50, 256–267.

Lundqvist, H., 1980. Influence of photoperiod on growth in Baltic salmon parr (*Salmo salar* L.) with special reference to the effect of precocious sexual maturation. Canadian Journal of Zoology 58, 940–944.

MacCrimmon, H.R., Campbell, J.S., 1969. World distribution of brook trout, *Salvelinus fontinalis*. Journal of the Fisheries Research Board of Canada 26, 1699–1725.

Mayer, I., Bornestaf, C., Borg, B., 1997. Melatonin in non-mammalian vertebrates: physiological role in reproduction? Comparative Biochemistry and Physiology Part A: Physiology 118, 515–531.

McCormick, S.D., Naiman, R.J., 1984. Some determinants of maturation in brook trout, *Salvelinus fontinalis*. Aquaculture 43, 269–278.

Meyer, K.A., Lamansky Jr., J.A., Schill, D.J., 2006. Evaluation of an unsuccessful brook trout electrofishing removal project in a small Rocky Mountain stream. North American Journal of Fisheries Management 26, 849–860.

Migaud, H., Fontaine, P., Sulistyo, I., Kestemont, P., Gardeur, J.N., 2002. Induction of out-of-season spawning in Eurasian perch *Perca fluviatilis*: effects of rates of cooling and cooling durations on female gametogenesis and spawning. Aquaculture 205, 253–267.

Migaud, H., Davie, A., Taylor, J., 2010. Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. Journal of Fish Biology 76, 27–68.

Munro, A.D., 1990. Tropical freshwater fish. In: Munro, A., Scott, A., Lam, T. (Ed.), Reproductive Seasonality in Teleosts: Environmental Influences. CRC Press, Florida, pp. 145–239.

Naiman, R.J., McCormick, S.D., Montgomery, W.L., Morin, R., 1987. Anadromous brook charr, *Salvelinus fontinalis*: Opportunities and constraints for population enhancement. Marine Fisheries Review 49, 1–13.

Naylor, R.L., Goldburg R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017–1024.

Noori, A., Mojazi Amiri, B., Mirvaghefi, A., Rafiee, G., Kalvani Neitali, B., 2015. Enhanced growth and retarded gonadal development of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum) following a long-day photoperiod. Aquaculture Research 46, 2398–2406.

Okuzawa, K., 2002. Puberty in teleosts. Fish Physiology and Biochemistry 26, 31–41.

Oppedal, F., Berg, A., Olsen, R.E., Taranger, G.L., Hansen, T., 2006. Photoperiod in seawater influence seasonal growth and chemical composition in autumn sea-transferred Atlantic salmon (*Salmo salar* L.) given two vaccines. Aquaculture 254, 396–410.

Pankhurst, N., Porter, M., 2003. Cold and dark or warm and light: variations on the theme of environmental control of reproduction. Fish Physiology and Biochemistry 28, 385–389.

Pankhurst, N., King, H., 2010. Temperature and salmonid reproduction: implications for aquaculture. Journal of Fish Biology 76, 69–85.

Peter, R.E., Yu, K.L., 1997. Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. Reviews in Fish Biology and Fisheries 7, 173–197.

Porter, M.J.R., Duncan, N., Handeland, S.O., Stafansson, S.O., Bromage, N.R., 2001. Temperature, light intensity and plasma melatonin levels in juvenile Atlantic salmon. Journal of Fish Biology 58, 431–438.

Power, G., 1980. The brook trout, *Salvelinus fontinalis*. In: Balon E.K. (Ed.), Charrs: Salmonid fishes of the genus *Salvelinus*. Dr W. Junk, The Hague, the Netherlands, pp. 141–203.

Prat, F., Zanuy, S., Bromage, N., Carrillo, M., 1999. Effects of constant short and long photoperiod regimes on the spawning performance and sex steroid levels of female and male sea bass. Journal of Fish Biology 54, 125–137.

Purchase, C., Boyce, D., Brown, J., 2000. Growth and survival of juvenile yellowtail flounder *Pleuronectes ferrugineus* (Storer) under different photoperiods. Aquaculture Research 31, 547–552.

Raleigh, R.F., 1982. Habitat suitability index models: brook trout. Western Energy and Land Use Team, Fish and Wildlife Service, U.S. Department of the Interior, Washington, DC, 42 pp.

Randall, C., Bromage, N., Thorpe, J., Miles, M., Muir, J., 1995. Melatonin rhythms in Atlantic salmon (*Salmo salar*) maintained under natural and out-of-phase photoperiods. General and Comparative Endocrinology 98, 73–86.

Randall, C., North, B., Futter, W., Porter, M., Bromage, N., 2001. Photoperiod effects on reproduction and growth in rainbow trout. Trout News 32, 12–15.

Rodrıguez, L., Zanuy, S., Carrillo, M., 2001. Influence of daylength on the age at first maturity and somatic growth in male sea bass (*Dicentrarchus labrax*, L.). Aquaculture 196, 159–175.

Sébert, M.E., Legros, C., Weltzien, F.A., Malpaux, B., Chemineau, P., Dufour, S., 2008. Melatonin activates brain dopaminergic systems in the eel with an inhibitory impact on reproductive function. Journal of Neuroendocrinology 20, 917–929.

Schulz, R., Andersson, E., Taranger, G., 2006. Photoperiod manipulation can stimulate or inhibit pubertal testis maturation in Atlantic salmon (*Salmo salar*). Animal Reproduction 3, 121–126.

Sloat, M.R., Fraser, D.J., Dunham, J.B., Falke, J.A., Jordan, C.E., McMillan, J.R., Ohms, H.A., 2014. Ecological and evolutionary patterns of freshwater maturation in Pacific and Atlantic salmonines. Reviews in Fish Biology and Fisheries 24, 689–707.

Stefansson, S.O., Björnsson, B.T., Hansen, T., Haux, C., Taranger, G.L., Saunders, R.L., 1991. Growth, parr-smolt transformation, and changes in growth hormone of Atlantic salmon (*Salmo salar*) reared under different photoperiods. Canadian Journal of Fisheries and Aquatic Sciences 48, 2100–2108.

Sundararaj, B., Vasal, S., 1976. Photoperiod and temperature control in the regulation of reproduction in the female catfish. Journal of the Fisheries Research Board of Canada 33, 959–973.

Taranger, G.L., Haux, C., Hansen, T., Stefansson, S.O., Björnsson, B.T., Walther, B.T., Kryvi, H., 1999. Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. Aquaculture 177, 47–60.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.-A., Dufour, S., Karlsen, Ø., Norberg, B., Angersson, E., Hansen, T., 2010. Control of puberty in farmed fish. General and Comparative Endocrinology 165, 483–515.

Taylor, J.F., Porter, M.J.R., Bromage, N.R., Migaud, H., 2008. Relationships between environmental changes, maturity, growth rate and plasma insulin-like growth factor-I (IGF-I) in female rainbow trout. General and Comparative Endocrinology 155, 257–270.

Thorpe, J., 2004. Life history responses of fishes to culture. Journal of Fish Biology 65, 263–285.

Thorpe, J.E., 2007. Maturation responses of salmonids to changing developmental opportunities. Marine Ecology Progress Series 335, 285–288.

Unwin, M., Rowe, D., Poortenaar, C., Boustead, N., 2005. Suppression of maturation in 2-year-old Chinook salmon (*Oncorhynchus tshawytscha*) reared under continuous photoperiod. Aquaculture 246, 239–250.

Vera, L., Davie, A., Taylor, J., Migaud, H., 2010. Differential light intensity and spectral sensitivities of Atlantic salmon, European sea bass and Atlantic cod pineal glands *ex vivo*. General and Comparative Endocrinology 165, 25–33.

Wydoski, R.S., Whitney, R.R., 2003. Inland fishes of Washington. American Fisheries Society, 384 pp.

CHAPTER 2

THE EFFECTS OF A PROLONGED PHOTOPERIOD AND LIGHT SOURCE ON GROWTH, SEXUAL MATURATION, FIN CONDITION, AND VULNERABILITY TO FUNGAL DISEASE IN BROOK TROUT *SALVELINUS FONTINALIS*

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The effects of a prolonged photoperiod and light source on growth, sexual maturation, fin condition, and vulnerability to fungal disease in brook trout *Salvelinus fontinalis*

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Abstract

The effects of an artificially prolonged photoperiod on growth, survival rate, colouration, and sexual maturation in brook trout *Salvelinus fontinalis* during pre- and post-spawning periods from 21 June to 06 November were investigated. Fish of mean initial weight ~150 g were reared at ambient photoperiod as well as with an artificially prolonged photoperiod produced by either a light-emitting diode or a metal-halide light. The fish groups subjected to a prolonged period of artificial light grew significantly larger and had a higher survival rate ($p < 0.05$), regardless of sex, and showed lower occurrence of fungal disease compared to controls reared in the natural photoperiod. We found a significantly higher number of sexually mature fish in the control groups compared with experimental groups. The increased photoperiod effectively delayed gonad development and increased somatic growth in both male and female brook trout, and also increased resistance to fungal disease. The increased photoperiod produced an observable difference in fish colouration, with control groups exhibiting more intensive spawning colouration.

KEYWORDS
growth, light source, photoperiod, *Salvelinus fontinalis*, sexual maturation

1 | INTRODUCTION

Sexual maturation is an energy-costly period in the life cycle of most fish, including brook trout *Salvelinus fontinalis* Mitchill. It can be characterized by reduced growth, increased aggression of males, deterioration of flesh quality, and increased susceptibility to disease (Sacobie, Burke, Lall, & Benfley, 2016). Since liver and muscle are the main energy suppliers for gonad growth and spawning (Dambergs, 1964; Kjesbu, Klungsøyr, Kryvi, Witthames, & Greer Walker, 1991), during spawning period growth performance relative to immature fish is reduced. Because of the negative influence of the spawning period, delaying age of sexual maturation can be an element of environmentally sustainable and economical aquaculture (Karlsen, Norberg, Kjesbu, & Taranger, 2006).

Sexual maturation in teleost fish is controlled by endogenous rhythms (Bromage, Porter, & Randall, 2001) that are synchronized by environmental cues (Zeitgebers). Photoperiod is an important factor that elicits synchronous spawning in salmonids (Skjæraasen et al., 2004), and its manipulation in several species of Salmonidae has shown to affect reproduction, feeding behaviour, and growth rate without negative consequences (Boeuf & Bail, 1999; Brown, Baumann, & Conover, 2014; Saunders, Henderson, & Harmon, 1985). Artificial photoperiod extension can be used to increase production efficiency and provide year-round reproduction (Önder, Başçınar, Khan, & Sonay, 2016).

The brook trout is a highly adaptable and relatively hardy (Jobling et al., 2010) North American salmonid that is becoming popular in Central and North Europe fish farming (Svinger et al., 2013) because of its suitability for flow-through and recirculating aquaculture systems and its high palatability (Zajic, Mraz, Sampels, & Pickova, 2016). The general time of spawning for this species is seasonal, namely in the autumn (Holcombe, Pasha, Jensen, Tietge, & Ankley, 2000). However, a number of factors affect the specific spawning time, principally photoperiod (Warren, Robinson, Josephson, Sheldon, & Kraft, 2012). Several studies have reported that brook trout shows promise for intensive aquaculture (Amin, Carter, Katersky Barnes, & Adams, 2016; Rasmussen & Ostenfeld, 2000), but commercial breeding is limited due to lack of knowledge of its biology, digestive function, diet requirements, and optimal technology for captive breeding (Önder et al., 2016).

The primary aim of this study was to investigate the effect of a prolonged photoperiod and the light source on the sexual maturation, growth rate, and overall performance of brook trout. Secondary aims were (a) to evaluate effect of light source and prolonged photoperiod on fish colouration and (b) to evaluate effect of light source and prolonged photoperiod on susceptibility of fish to secondary fungal disease during transport.

2 | MATERIALS AND METHODS

2.1 | Fish and rearing conditions

Salvelinus fontinalis (n = 2,700) of mean initial body weight 100–150 g were randomly distributed among nine outdoor concrete flow-through tanks (3.6 m^3, 720 × 100 cm) with water flow rate 5.1–5.7 m^3/h. Stocking density was 8.5–10 kg/m^3. All tanks were equipped with a lighting system consisting of three lamps located above the tank, either light-emitting diodes (LED) or metal-halide (HAL) lights. The metal-halide source consisted of a Panlux V500/C lamp with OSRAM HALOLINE ECO bulbs (400 W, 9,000 lm, 2,950 K). The LED light sources used are Epistar 50 W LED COB lamps (50 W, 4,000 lm, 6,000 K). The light intensity at half the height of the water column was approx. 1,400–100 lx depending on the distance from the light source. This light intensity was the same for both light sources.

Three groups of fish (three tanks per group) under different light regimes were tested: HAL—ambient light photoperiod from 24 April to 21 June, with the light period extended to 18 hr using metal-halide lamps from 21 June to 06 November; LED—ambient light photoperiod from 24 April to 21 June, with the light period extended to 18 hr using LED lamps from 21 June to 06 November; and AMB—natural ambient photoperiod throughout the study. Artificial lighting was used at evening started from 1 hour before sunset for prolongation of photoperiod to 18 hr of daylight. A dimmer was used to reduce light intensity from daylight to darkness during the final hour of the light period.

Fish were fed Skretting Optiline HE granulated food (protein 41.5%–43.5%, fat 26.5%–28.5%, carbohydrates 15%–15.5%) by automatic belt feeders at rates recommended by the manufacturer, from 700 to 1700 hr. The daily ration was adjusted depending on the water temperature in accordance with the recommendations of the feed manufacturer. The quantity of feed consumed was monitored for subsequent calculation of feeding coefficient. The fish were not fed for 48 hr before collecting experimental data.

Monitoring of dissolved oxygen, temperature, pH, and fish mortality was carried out daily at 1,500 hr. Light intensity was measured by an underwater illumination meter (luxmeter) Hydrolux (BGB Innovation) at a point half the height of the water column, that is 25 cm above the bottom.

2.2 | Morphometrics and condition indices

Body weight (BW), total length (L_T), and standard length (L_s) were measured in 30 males and 30 females from each treatment on 21 June, 13 August, 08 October, and 06 November, corresponding to 0, 53, 109, and 138 days post-midsummer (Figure 1). Separation of males and females was carried out based on the exterior principles—colouration and length of the lower jaw. After killing with an overdose of anaesthetic, fish were dissected and gonads removed for further investigation. Samples of gonads, liver, spleen, and perivisceral fat were fixed in Bouin's solution. Perivisceral fat was collected manually from all possible tissues and organs. Growth indices were determined as follows:

$$\text{Condition factor}(K) = 100 \times \tilde{}\text{BW} \times L_T^{\tilde{}-3}$$

$$\text{Hepatosomatic index}(HSI) = 100\tilde{} \times \tilde{}\text{liver weight}/\text{BW}$$

$$\text{Gonadosomatic index}(GSI) = 100\tilde{} \times \text{gonad weight}/\text{BW}$$

$$\text{Perivisceral fat index}(PVSI) = 100 \times \tilde{}\text{fat weight}/\text{BW}$$

$$\text{Spleenosomatic index}(SSI) = 100\tilde{} \times \tilde{}\text{spleen weight}/\text{BW}$$

2.3 | Fin condition

In connection with the results of previous studies of increased male aggressivity during the spawning period, we decided to assess fin condition. On 21 June and 06 November (midsummer and 138 days after midsummer), 100 fish per tank were anaesthetized with 0.03 ml/l clove oil, and total body length, standard length, and length of fins were measured to 1 mm to calculate relative fin length (RFL). As an indicator of overall degree of fin damage, we calculated the TRFL, which represents

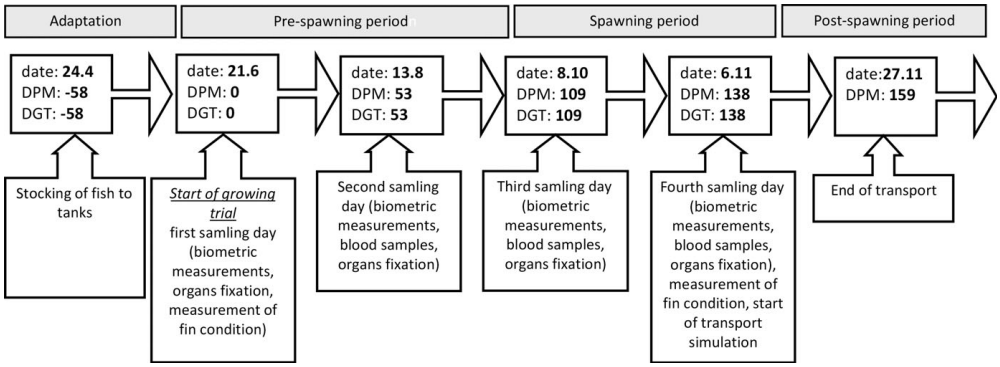

FIGURE 1 Experimental design. DPM: days post-midsummer (midsummer = summer solstice); DGT: days of growth trial

the ratio of the total length of all fins to the total length of the body (Stejskal, Policar, Kristian, Kouril, & Hamackova, 2011).

$$\mathrm{RFL} = \mathrm{TFL}/\mathrm{SL} \times 100$$

$$\mathrm{TRFL} = (\mathrm{LAF} + \mathrm{LDF} + \mathrm{LCF} + \mathrm{LRPLF} + \mathrm{LLPLF} + \mathrm{LRPTF} + \mathrm{LLPTF})\,\mathrm{SL}^{-1}$$

In which TRFL: total relative fin length; RFL: relative fin length; TFL: total fin length; SL: standard length; LAF: length of anal fin; LDF: length of dorsal fin; LCF: length of caudal fin; LRPLF: length of right pelvic fin; LLPLF: length of left pelvic fin; LRPTF: length of right pectoral fin; and LLPTF: length of left pectoral fin.

2.4 | Plasma sex hormones and glucose level

All blood samples were taken from tail veins. The plasma separation from the blood was performed using a standard technique using a centrifuge (10 min, 4°C, 12,000 × *g*).

Plasma glucose level was analysed on an Architect 8,000 chemistry analyser (Abbott Laboratories, USA) and hormones on the Immulite 2000XPi immunoassay system (Siemens).

Glucose analyses used the hexokinase/G-6-PDH spectrophotometric assay. Oestradiol and testosterone levels were determined by solid-phase, enzyme-labelled chemiluminescent competitive immunoassays. Two microlitres of glucose, 25 µl of oestradiol, and 20 µl of testosterone were used for analysis.

2.5 | Evaluation of colour

Detailed analyses are needed to determine the relationship between sexual maturation and fish colour. These data can be effectively used in determining the term of spawning without creating unnecessary stress to fish. For this purpose, an analysis of fish colouration was carried out. The fish were photographed according to their groups (light source and sex). The camera was calibrated using the white balance to normalize light conditions and provide as accurate colour interpretation as possible. The group images were processed and analysed by Fish Skin Colour Evaluation Application software [1], Urban/, 2011/2012, 2011/2012, 2011/2012. The application consists of several steps: (a) fish location in the image, (b) colour transformation (Figure 2), and (c) statistical evaluation.

To evaluate only the colour information from the fish body, the image is transformed from camera native RGB representation into chromatic colours (brightness and shadows removed) and segmented using maximization of variance between the fish and background pixels.

The fish body is transformed into a mathematical colour representation (colour spaces), including the hue saturation value and CIE L*a*b*, the most commonly used representations for colour comparison. The hue channel represents the distribution of the colour across the fish body (Urban, 2017).

For the statistical analysis, only significant differences ($p < 0.05$) in colour distribution among groups were considered. All analysis was done automatically.

2.6 | Simulation of transport from farm and storage before slaughter with respect to fungal disease

From 06 November to 27 November, a 21-day experiment was conducted to evaluate the susceptibility of brook trout grown under different light conditions to secondary fungal infection (saprolegniosis). The aim of this trial was to simulate live fish transport in water with hydrochemical parameters different from the rearing system and to assess occurrence of secondary fungal disease. Eighteen hundred fish were transported approximately 90 km to another fish breeding farm and divided among 27 plastic tanks (3 m^3, 3 × 1 × 0.9 m). Each treatment group had its own special transportation container. Transportation was carried out at the same time, same vehicle during 1 day.

Nine groups of fish were tested in triplicate: Hf/m: fish reared under HAL lighting, sex ratio 1:1; Hf: females reared with HAL lighting; Hm: males reared under HAL lighting; Lf/m: fish reared with LED lighting, sex ratio 1:1; Lf: females reared under LED lighting;

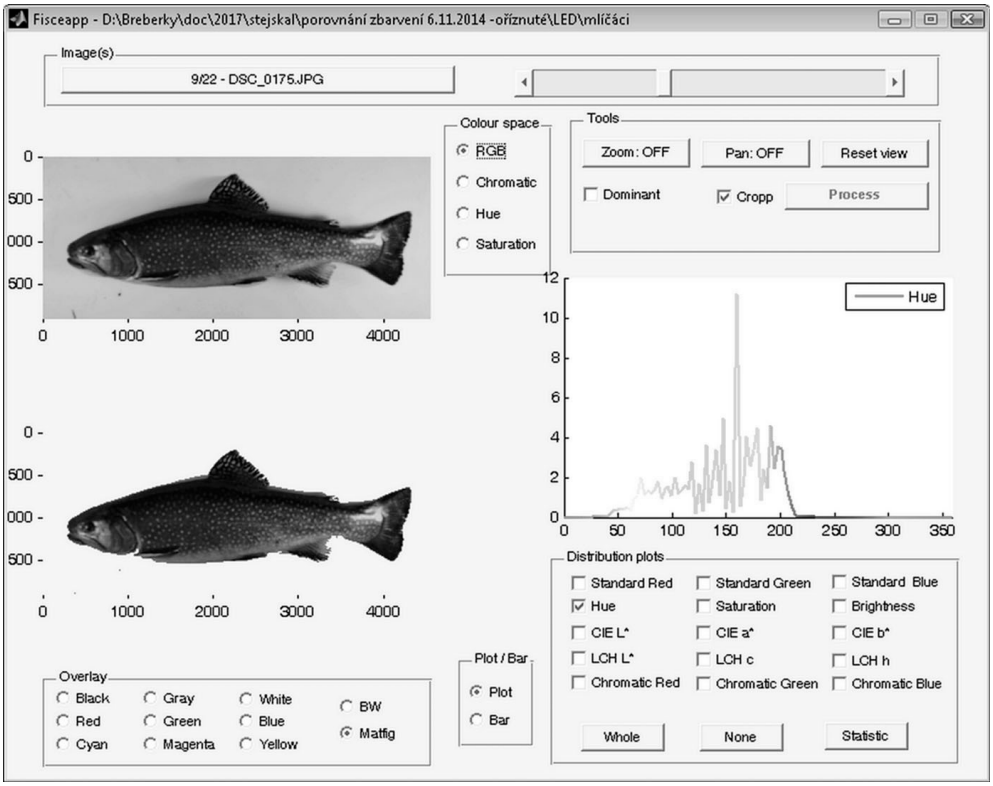

FIGURE 2 Image analysis using normalization of the light conditions and segmentation of image via maximization of inter-class variation, to locate the fish in the image scene [Colour figure can be viewed at wileyonlinelibrary.com]

Lm: males reared under LED lighting; AMBf/m: fish reared in natural light, sex ratio 1:1; AMBf: females reared in natural light; AMBm: males reared in natural light.

Visual evaluation of fungal disease prevalence was carried out every 7 days. Fish mortality was monitored daily.

$$\text{Daily losses}\,(\%) = 100/(N_s/N_d)$$

$$\text{Survival}\,(\%) = 100/N_s \times N_c$$

$$\text{Prevalence of fungal infection}\,(\%) = (100/N_c \times N_i)$$

in which N_s: number of stocked fish, N_d: number of dead fish, N_c: number of surviving fish, and N_i: number of fish with fungal infection.

2.7 | Economic analysis

The economic conversion ratio (ECR) and economic profit index (EPI) for each group was calculated using modified formulae of Moutinho et al. (2017) to determine the relative efficiency of treatments as follows:

$$\text{ECR} = \text{FCR} \times [\text{DP} + (\text{EC}/\text{B})]$$

where ECR: economic conversion ratio (€ per kg fish); FCR: feed conversion ratio (kg diet/kg fish); DP: diet price (€/kg); EC: cost of energy (€); B: biomass (kg).

$$\text{EPI} = (\text{WG} \times \text{SP}) - (\text{WG} \times [\text{DP} + (\text{EC}/\text{B})])$$

where EPI: economic profit index (€/fish); WG: weight gain (kg); SP: selling price (5 €/kg); DP: diet price (€/kg); EC: cost of energy (€); B: biomass (kg).

2.8 | Statistical analysis

Data were analysed with Microsoft Excel 2010 (Microsoft, Inc., USA). Statistical analysis consisted of a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (Statistica 12.0; StatSoft, Inc., USA). When the ANOVA assumptions were not satisfied, the

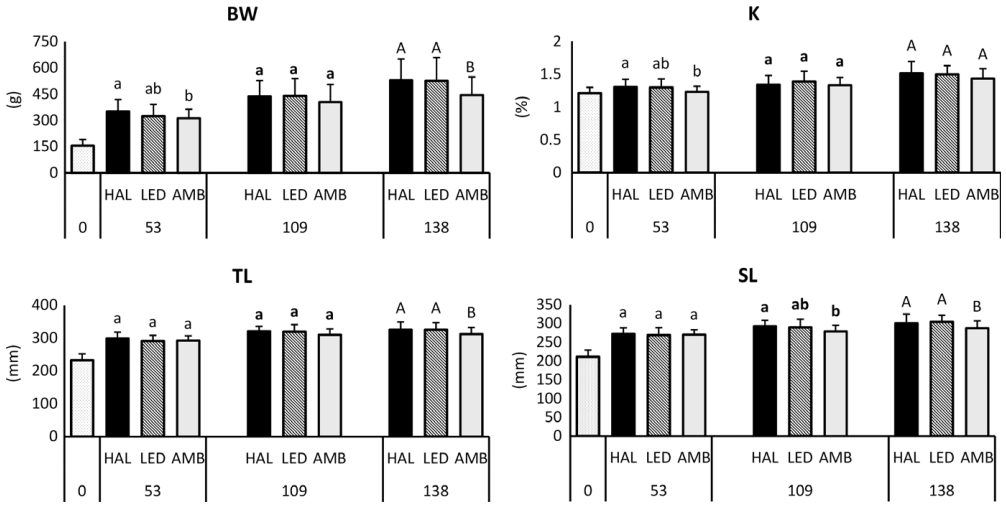

FIGURE 3 Male brook trout body weight (BW, g), condition factor (K), total length (TL, mm), and standard length (SL, mm) with different photoperiods and light sources. Data are shown as mean (bars) ± *SD* (whiskers). Different letters above bars indicate significant difference among treatments at a sampling date ($p < 0.05$)

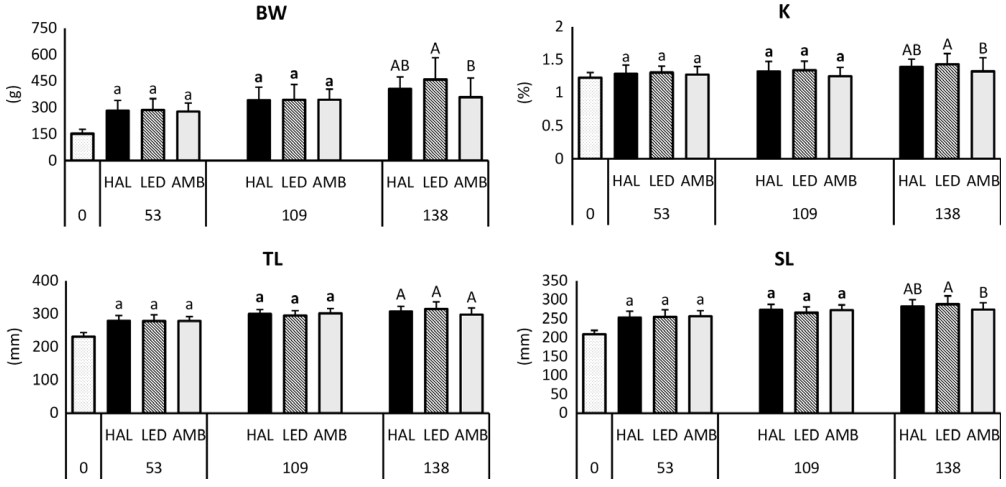

FIGURE 4 Female brook trout body weight (BW, g), condition factor (K), total length (TL, mm), and standard length (SL, mm) with different photoperiods and light sources. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$)

differences between groups were tested using the nonparametric Kruskal–Wallis test. The level of significance chosen for all analyses was $p < 0.05$. The statistical analysis of fish colouration and fish group comparison used central moments and one-way ANOVA test with significance above 95% ($p < 0.05$).

3 | RESULTS

The mean initial BW at the start of the study was 152.7 ± 24.3 g for females and 155.9 ± 34.4 g for males. At the conclusion of the trial, the AMB groups showed significantly lower mean BW and SL

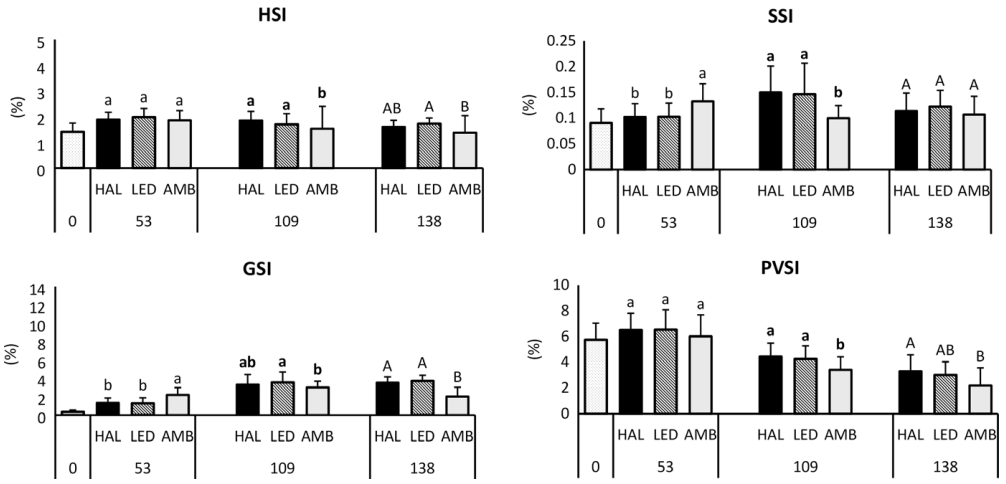

FIGURE 5 Male brook trout hepatosomatic index (HSI, %), gonadosomatic index (GSI, %), perivisceral fat index (PVSI, %), and spleenosomatic index (SSI, %) with different photoperiods and light sources. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$)

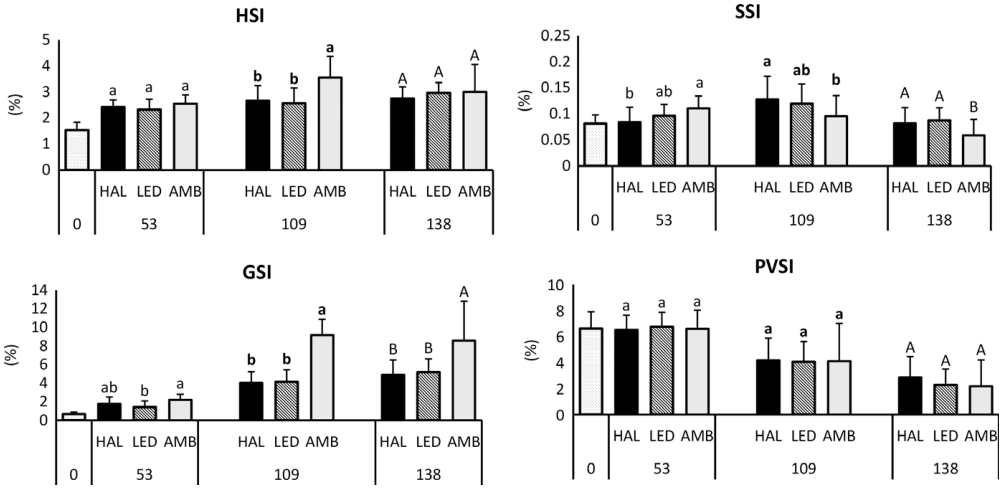

FIGURE 6 Hepatosomatic index (HSI, %), gonadosomatic index (GSI, %), perivisceral fat index (PVSI, %), and splenosomatic index (SSI, %) of female brook trout reared at different photoperiods and light sources. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$)

in both sexes (Figures 3 and 4). On day 138, AMB group males showed significantly lower TL (312.3 ± 20.3 mm) compared to all other treatments (Figure 3).

On day 138, the mean K of females was significantly higher in the LED group (1.4 ± 0.16) than in AMB (Figure 4). Significantly higher mean K was observed in HAL males (1.3 ± 0.12) compared to AMB on day 53 (Figure 3). Feed conversion ratio reached levels 1.14 ± 0.05, 1.11 ± 0.01, and 1.13 ± 0.04 for HAL, LED, and AMB group respectively.

The mean HSI of males was significantly lower in the AMB group compared with all other groups on day 109, and with LED lighting at the end of experiment (1.5 ± 0.9 and 1.4 ± 0.7, respectively)

TABLE 1 Male brook trout relative fin length (cm) with respect to light source

	day 0			day 138		
	CON	HAL	LED	CON	HAL	LED
DF/TL	0.097 ± 0.018^{ab}	0.100 ± 0.022^{a}	0.094 ± 0.025^{b}	0.106 ± 0.091^{A}	0.098 ± 0.013^{B}	0.097 ± 0.011^{B}
CF/TL	0.112 ± 0.009^{b}	0.110 ± 0.007^{b}	0.118 ± 0.012^{a}	0.112 ± 0.010^{A}	0.102 ± 0.011^{B}	0.107 ± 0.010^{AB}
AF/TL	0.104 ± 0.009^{a}	0.104 ± 0.009^{a}	0.108 ± 0.015^{a}	0.120 ± 0.011^{A}	0.117 ± 0.021^{A}	0.113 ± 0.008^{B}
RPLF/TL	0.098 ± 0.007^{ab}	0.096 ± 0.006^{b}	0.100 ± 0.013^{a}	0.111 ± 0.009^{A}	0.105 ± 0.009^{B}	0.107 ± 0.007^{AB}
LPLF/TL	0.097 ± 0.007^{ab}	0.096 ± 0.006^{b}	0.101 ± 0.013^{a}	0.111 ± 0.010^{A}	0.105 ± 0.008^{B}	0.107 ± 0.007^{AB}
RPTF/TL	0.094 ± 0.023^{a}	0.100 ± 0.015^{a}	0.100 ± 0.024^{a}	0.120 ± 0.023^{A}	0.120 ± 0.014^{A}	0.115 ± 0.022^{A}
LPTF/TL	0.095 ± 0.020^{a}	0.099 ± 0.012^{a}	0.100 ± 0.020^{a}	0.120 ± 0.026^{A}	0.118 ± 0.015^{AB}	0.115 ± 0.013^{B}
TRFL	0.70 ± 0.052^{a}	0.71 ± 0.039^{a}	0.72 ± 0.083^{a}	0.80 ± 0.055^{A}	0.77 ± 0.041^{B}	0.76 ± 0.036^{B}

Note. Data are shown as mean ± *SD*.
AF: anal fin; CF: caudal fin; DF: dorsal fin; LPLF: left pelvic fin; LPTF: left pectoral fin; RPLF: right pelvic fin; RPTF: right pectoral fin; TL: total length; TRFL: total relative fin length.
Within-sampling day values with different superscripts are significantly different ($p < 0.05$).

TABLE 2 Female brook trout relative fin length (cm) with respect to light source

	day 0			day 138		
	CON	HAL	LED	CON	HAL	LED
DF/TL	0.095 ± 0.020^{a}	0.102 ± 0.018^{a}	0.095 ± 0.017^{a}	0.102 ± 0.011^{A}	0.095 ± 0.011^{A}	0.095 ± 0.013^{A}
CF/TL	0.113 ± 0.008^{b}	0.111 ± 0.007^{b}	0.117 ± 0.008^{a}	0.113 ± 0.011^{A}	0.100 ± 0.010^{B}	0.101 ± 0.008^{B}
AF/TL	0.106 ± 0.009^{a}	0.105 ± 0.008^{a}	0.106 ± 0.010^{a}	0.123 ± 0.011^{A}	0.117 ± 0.014^{AB}	0.111 ± 0.010^{B}
RPLF/TL	0.095 ± 0.006^{b}	0.094 ± 0.006^{b}	0.100 ± 0.008^{a}	0.105 ± 0.006^{A}	0.102 ± 0.008^{A}	0.100 ± 0.008^{A}
LPLF/TL	0.095 ± 0.007^{b}	0.094 ± 0.006^{b}	0.100 ± 0.008^{a}	0.105 ± 0.006^{A}	0.102 ± 0.008^{A}	0.101 ± 0.008^{A}
RPTF/TL	0.098 ± 0.014^{a}	0.100 ± 0.011^{a}	0.099 ± 0.016^{a}	0.120 ± 0.011^{A}	0.117 ± 0.015^{A}	0.106 ± 0.014^{B}
LPTF/TL	0.098 ± 0.015^{a}	0.101 ± 0.009^{a}	0.104 ± 0.009^{a}	0.117 ± 0.022^{A}	0.117 ± 0.014^{AB}	0.109 ± 0.015^{B}
TRFL	0.70 ± 0.042^{b}	0.71 ± 0.036^{ab}	0.72 ± 0.043^{a}	0.79 ± 0.049^{A}	0.75 ± 0.047^{A}	0.72 ± 0.049^{A}

Note. Data are shown as mean ± *SD*.
AF: anal fin; CF: caudal fin; DF: dorsal fin; LPLF: left pelvic fin; LPTF: left pectoral fin; RPLF: right pelvic fin; RPTF: right pectoral fin; TL: total length; TRFL: total relative fin length.
Within-sampling day values with different superscripts are significantly different ($p < 0.05$).

(Figure 5). The opposite tendency was exhibited in females, in which HSI on day 109 was highest ($p < 0.05$) in the AMB group (3.6 ± 0.8) (Figure 6). At the end of experiment, significantly lower mean SSI (0.06 ± 0.03) was observed in the AMB females compared to prolonged lighting treatments (Figure 6). The lowest level ($p < 0.05$) of SSI and PVSI in males was in the AMB group (3.4 ± 0.99 and 0.1 ± 0.02, respectively) on day 109 compared to the other treatments. At day 138, significantly higher PVSI was seen in HAL group males (3.3 ± 1.3) than in the AMB group (Figure 5). On the final two testing dates, GSI differed according to sex, with females from AMB showing significantly higher GSI compared to other experimental groups, while males of the AMB group presented lowest mean GSI ($p < 0.05$).

Both sexes showed significant differences in RFL, with the longest fins at the conclusion of the trial found in the controls (Tables 1 and 2). Fin lesions were minimal.

On day 53, significantly lower glucose among male groups was observed with the HAL treatment. At day 109 significantly lower glucose was seen in LED compared to AMB ($p < 0.05$). Among females, the lowest glucose level was observed in AMB at the end of experiment ($p < 0.05$). The AMB females showed significantly higher testosterone than other female groups throughout the trial, while oestradiol levels changed over the monitored period. At prespawning and until the middle of the spawning period (Figure 1), the AMB group showed the highest level of oestradiol ($p < 0.05$). At completion of the trial, a significantly higher level was observed in HAL compared to LED. In males on day 53, AMB showed significantly higher levels of oestradiol than HAL ($p < 0.05$) (Figure 7).

In the colour analysis, the average hue distribution of males in the AMB group differed from HAL and LED by 48% and 41% respectively. A difference of 22% was found between HAL and LED, with slight homogenization (lower *SD*) and a shift to green hue observed in HAL.

Among females, a similar difference was found among light treatments, with the AMB group distribution differing from HAL and LED by 41% and 29% respectively. The halogen lighting resulted in slight homogenization of hue along the fish body, shifting the colour towards blue (Figure 8).

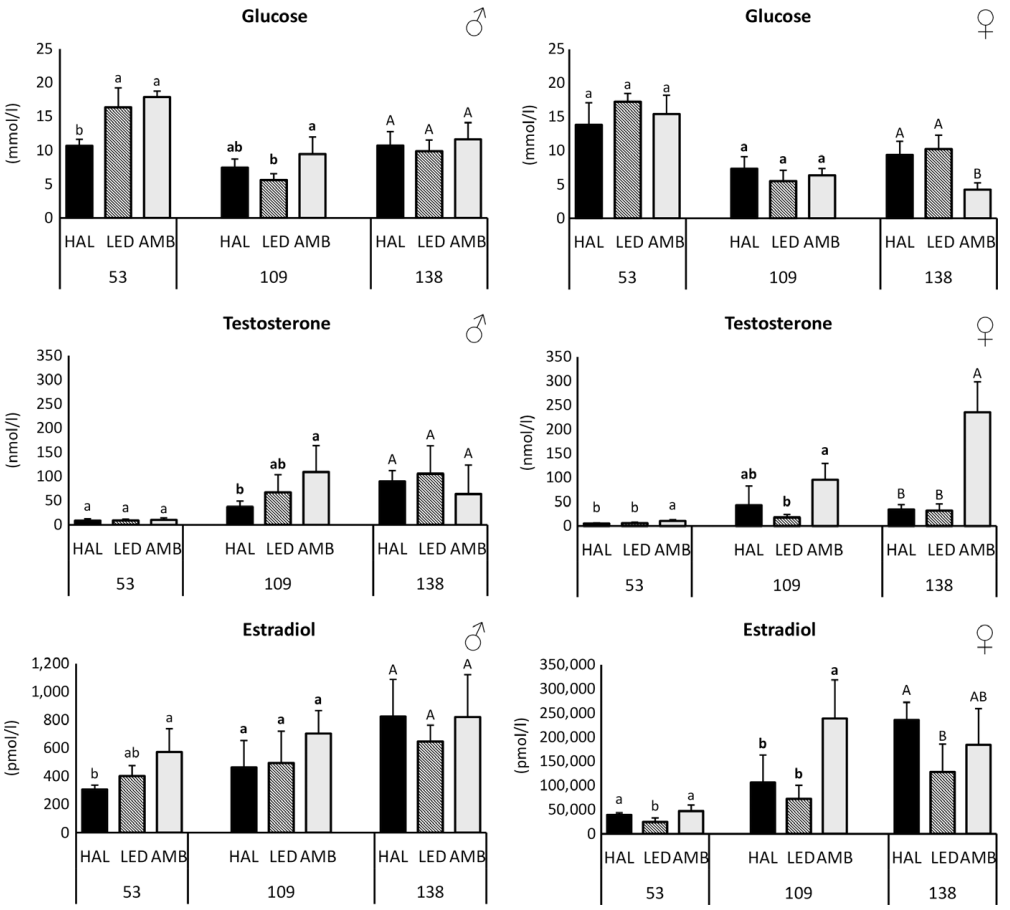

FIGURE 7 Temporal changes in glucose, testosterone, and oestradiol concentrations in blood plasma of female and male brook trout. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$)

At sampling times, fish were tested for spawning readiness by manual expression of gametes (Figure 9). On day 109, sexually mature fish were identified only in the male AMB group. At the conclusion of the trial, almost all control males and 41% of control females were mature. A considerably lower percentage of sexually mature individuals was seen in LED and HAL male groups (4% and 7%, respectively). No females from HAL and LED were found to have matured. Control groups had matured within the established period of spawning in the natural environment under optimal conditions.

At the end of this main experimental period, survival rate in all treatments had not dropped below 99%.

In the fish transportation and holding trial, the artificially extended photoperiod was found to significantly affect fish survival and occurrence of secondary fungal disease. Significantly, higher numbers of infected fish were found in control groups ($p < 0.05$) (Figure 10). Final survival after transport was higher ($p < 0.05$) in groups reared under the prolonged photoperiod, regardless of the light source or sex (Figure 11).

Significantly higher ($p < 0.05$) ECR was found in the HAL group, 2.9 ± 0.16 compared to the LED and AMB treatments at 1.8 ± 0.04 and 1.7 ± 0.06 respectively. The opposite situation was observed with EPI, with LED (557.9 ± 10.2) and AMB (572.2 ± 21.8) showing significantly higher indices than HAL (478.1 ± 23.5).

4 | DISCUSSION

Photoperiod manipulation is an environmentally acceptable method for controlling timing of maturation and spawning in salmonids

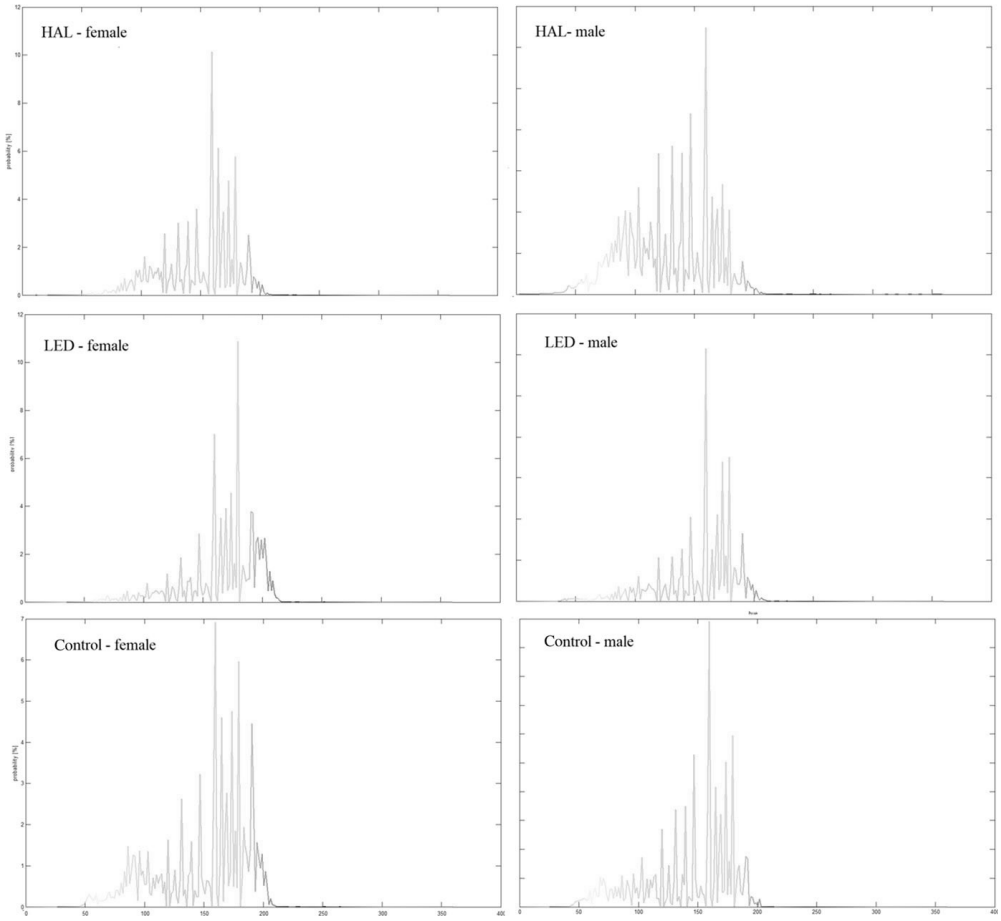

FIGURE 8 Computer analysis of colouration and colour transformation of brook trout; distribution of hue values according to FISCEAPP software [Colour figure can be viewed at wileyonlinelibrary.com]

(Bromage, Randall, Dunston, Thrush, & Jones, 1993; Holcombe et al., 2000), and, consequently, influencing growth (Endal, Taranger, Stefansson, & Hansen, 2000). A growth-promoting effect of a prolonged photoperiod was seen in the present study, in accordance with previous research (Karlsen et al., 2006; Önder et al., 2016; Taylor, Migaud, Porter, & Bromage, 2005). During pre-spawning and the first part of spawning periods (Figure 1) there were no significant differences among treatments in growth parameters for either sex. The growth rate of groups receiving artificially prolonged light regimes was higher than control groups. In females, significant effect on growth was obtained with the use of LED lights. No differences with respect to artificial light source were found for males. The growth increase evident in artificial light groups during the spawning period in this study is in accordance with Hansen et al. (2001) and can be explained by delay of gonad development and spawning, thereby postponing or minimizing the reduced somatic growth normally associated with sexual maturation (Taranger, Aardal, Hansen, & Kjesbu, 2006). Groups under the prolonged light regime likely invested less energy in sexual maturation than did controls. This is supported by lower mean GSI values in females of the HAL and LED groups compared to AMB. Taranger et al. (2006) suggested that this may represent a stimulatory effect of a prolonged photoperiod on somatic growth, independent of sexual maturation, as was found in Atlantic salmon *Salmo salar* (Oppedal, Taranger, Juell, Fosseidengen, & Hansen, 1997). Male GSI altered depending on the phase in the spawning period. During the natural spawning period, GSI decreased in the AMB group and gradually increased in experimental treatments. This indicates a delay in sexual maturation. This may be correlated with

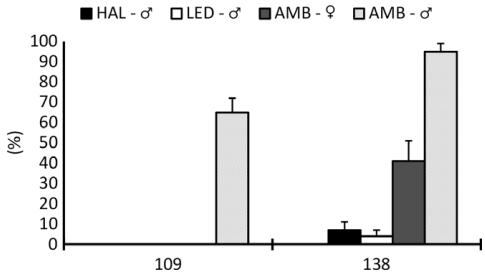

FIGURE 9 Spawning readiness of brook trout reared under different light sources. Data are shown as mean (bars) ± *SD* (whiskers)

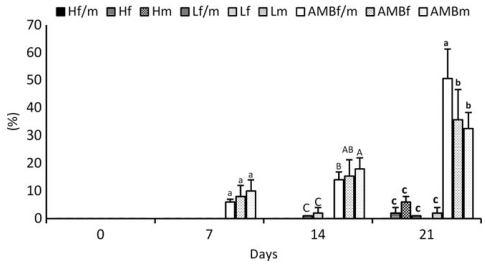

FIGURE 10 The prevalence of secondary fungal infection after transportation to other breeding facilities in brook trout reared under different photoperiod and light sources. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$)

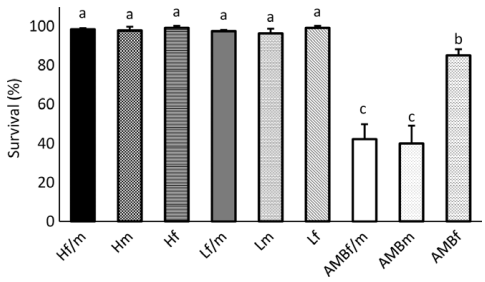

FIGURE 11 Final survival rate of brook trout reared under different photoperiods and light sources after transfer to other breeding facilities. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$)

the results of spawning readiness obtained at the conclusion of the trial. A significantly higher percentage of fish from control groups were mature, and no females of the experimental groups were ready for spawning.

Sex steroids play a significant role in regulation of fish gametogenesis, particularly in the synthesis of vitellogenin in the liver (Silversand, Hyllner, & Haux, 1993) and the proliferation of spermatogonia at the onset of spermatogenesis (Schulz et al., 2010). Seasonal variation in plasma oestrogen of brook trout is well-researched (Goetz, Fostier, Breton, & Jalabert, 1987; Tam, Roy, & Makaran, 1986), but information concerning sex hormone alterations associated with photoperiod manipulation is scarce. According to Tam et al. (1986), the highest oestradiol level in female brook trout is observed near the end of vitellogenesis. Subsequently, prior to ovulation, plasma oestradiol level is reduced. In the present study, the steeper increase in oestradiol levels in female controls compared to the other treatments, followed by increasing plasma oestradiol levels, indicated that photoperiod manipulation delayed maturation. These data are in agreement with studies on other salmonids, in which plasma oestradiol was higher in pre-ovulating females with reduction at the onset of ovulation (Mayer, Schmitz, Bergo, & Schulz, 1992; Pavlidis, Dimitriou, & Dessypris, 1994). In male brook trout, oestradiol plasma levels remained low at all sampling periods compared with females, in agreement with previous investigation of salmonids (Mayer et al., 1990). In the present study, testosterone levels in male brook trout were similar to those reported by Holcombe et al. (2000). Higher testosterone levels were recorded in females compared with males. This is consistent with Norberg et al. (1989) and Taranger et al. (1999). High concentrations of testosterone in females can be explained by the fact that testosterone is the precursor of oestradiol, and has a positive influence on gonadotropin production and aromatase activity (Callard, Schlinger, Pasmanik, & Corina, 1990; Dickhoff & Swanson, 1990). It may also be due to regulation of ovulatory cycles in batch spawners (Norberg, Brown, Halldorsson, Stensland, & Bjørnsson, 2004).

Changes in hue spectrum were similar for LED and HAL groups. The halogen lighting slightly decreased the standard deviation of the group; also there were more changes in the colouration ($p < 0.05$) in both males and females. The difference in colouration between AMB and experimental treatments was apparent in both sexes. Both male and female controls showed intense spawning colouration. However, to more accurately determine the reasons for the differences in fish colouration, specialized experimentation is needed.

Although saprolegniosis is not among the infections monitored by government programmes, it can lead to significant economic loss. Svobodova et al. (2007) suggested that it should be considered secondary to skin and gill injury. During the spawning period, salmonid males become more aggressive, which can lead to skin injury. Willoughby and Pickering (1997) reported that the sexual maturation is the most sensitive period for fish, and that stress reactions negatively affect the production of skin mucous, which is already reduced during sexual maturation in salmonids. We found the greatest number of fungus-infected fish in the AMB groups after transport (changes in environmental conditions), which also had a larger number of sexually mature fish. Significantly higher levels of infection in the control fish can be explained by increased aggression because of maturation as well as stress caused by transportation. This agrees

with data obtained by Pickering and Duston (1983) with respect to the role of stress in development of fungal disease in brown trout *Salmo trutta* L. We can also speculate (based on long-term observation of farmer) that brook trout does not display signs of secondary diseases at the grow-out unit because of lower pH and higher content of humic substances in water.

The artificially prolonged light period influenced the growth of brook trout regardless of sex. Based on results of this study, we can recommend LED lamps for an artificial lighting regime to postpone puberty. There was no difference in fish growth rate between the two light sources for the artificial prolonged photoperiod, but LED was more cost effective. The prolonged photoperiod was associated with higher survival rate of fish during transport and holding, reducing economic loss. Importantly, the extended light phase postponed sexual maturation in both sexes, which can be beneficial to commercial sales and produce year-round spawning. The results obtained will be useful for manipulation of maturation and spawning time of farmed brook trout to avoid deterioration in flesh quality, reduction in growth, and loss of fish, and to increase welfare. It is necessary to conduct research to determine optimal methods of implementation of the extended photoperiod in combination with other abiotic factors, such as temperature, feeding regime, water pH.

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REFERENCES

Amin, M. N., Carter, C. G., Katersky Barnes, R. S., & Adams, L. R. (2016). Protein and energy nutrition of brook trout (*Salvelinus fontinalis*) at optimal and elevated temperatures. *Aquaculture Nutrition*, *22*, 527–540.

Boeuf, G., & Le Bail, P.-Y. (1999). Does light have an influence on fish growth? *Aquaculture*, *177*, 129–152. https://doi.org/10.1016/S0044-8486(99)00074-5

Bromage, N. R., Porter, M., & Randall, C. (2001). The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture*, *197*, 63–98. https://doi.org/10.1016/S0044-8486(01)00583-X

Bromage, N., Randall, C., Dunston, J., Thrush, M., & Jones, J. (1993). Environmental control of reproduction in salmonids. In J. F. Muir, & R. J. Roberts (Eds.), *Recent advances in aquaculture*, Vol. *4* (pp. 55–65). Oxford, UK: Blackwell Scientific Publications.

Brown, E. E., Baumann, H., & Conover, D. O. (2014). Temperature and photoperiod effects on sex determination in a fish. *Journal of Experimental Marine Biology and Ecology*, *461*, 39–43. https://doi.org/10.1016/j.jembe.2014.07.009

Callard, G., Schlinger, B., Pasmanik, M., & Corina, K. (1990). Aromatization and estrogen action in brain. *Progress in Clinical and Biological Research*, *342*, 105–111.

Dambergs, N. (1964). Extractives of fish muscle. 4. Seasonal variations of fat, water-solubles, protein, and water in cod (*Gadus morhua* L.) fillets. *Journal of the Fisheries Research Board of Canada*, *21*, 703–709.

Dickhoff, W. W., & Swanson, P. (1990). Function of salmon pituitary glycoprotein hormones: "The maturational surge hypothesis". *Progress in Clinical and Biological Research*, *342*, 349–356.

Endal, H. P., Taranger, G. L., Stefansson, S. O., & Hansen, T. (2000). Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. *Aquaculture*, *191*, 337–349. https://doi.org/10.1016/S0044-8486(00)00444-0

Goetz, F. W., Fostier, A. Y., Breton, B., & Jalabert, B. (1987). Hormonal changes during meiotic maturation and ovulation in the brook trout (*Salvelinus fontinalis*). *Fish Physiology and Biochemistry*, *3*, 203–211. https://doi.org/10.1007/BF02180281

Hansen, T., Karlsen, Ø., Taranger, G. L., Hemre, G. I., Holm, J. C., & Kjesbu, O. S. (2001). Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture*, *203*, 51–67. https://doi.org/10.1016/S0044-8486(01)00610-X

Holcombe, G. W., Pasha, M. S., Jensen, K. M., Tietge, J. F., & Ankley, G. T. (2000). Effects of photoperiod manipulation on brook trout reproductive development, fecundity, and circulating sex steroid concentrations. *North American Journal of Aquaculture*, *62*, 1–11. https://doi.org/10.1577/1548-8454(2000)062<0001:EOPMOB>2.0.CO;2

Jobling, M., Arnesen, A. M., Benfey, T. J., Carter, C. G., Hardy, R. W., Le Francois, N. R., … Lamarre, S. G. (2010). The Salmonids. In N. R. Le Francois (Ed.), *Finfish aquaculture diversification* (pp. 234–289). UK: CAB International.

Karlsen, Ø., Norberg, B., Kjesbu, O. S., & Taranger, G. L. (2006). Effects of photoperiod and exercise on growth, liver size, and age at puberty in farmed Atlantic cod (*Gadus morhua* L.). *ICES Journal of Marine Science*, *63*, 355–364. https://doi.org/10.1016/j.icesjms.2005.10.013

Kjesbu, O. S., Klungsøyr, J., Kryvi, H., Witthames, P. R., & Greer Walker, M. (1991). Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*, 2333–2343.

Mayer, I., Lundqvist, H., Bergulund, I., Schmitz, M., Schulz, R., & Borg, B. (1990). Seasonal endocrine changes in Baltic salmon, *Salmo salar*, immature parr and mature male parr. I. Plasma levels of five androgens, 17a-hydroxy-20-b-dihydroprogesterone, and 17-b-estradiol. *Canadian Journal of Zoology*, *68*, 1360–1365.

Mayer, I., Schmitz, M., Bergo, B., & Schulz, R. (1992). Seasonal endocrine changes in male and female Arctic char (Salvelinus alpinus). I. Plasma levels of three androgens, 17a-hydroxy-20b-dihydroprogesterone, and 17b-estradiol. *Canadian Journal of Zoology*, *70*, 37–42.

Moutinho, S., Martinez-Llorens, S., Tomas-Vidal, A., Jovel-Cerda, M., Oliva-Teles, A., & Peres, H. (2017). Meat and bone meal as partial replacement for fish meal in diets for gilthead seabream (*Sparus aurata*) juveniles: Growth, feed efficiency, amino acid utilization, and economic efficiency. *Aquaculture*, *468*, 271–277. https://doi.org/10.1016/j.aquaculture.2016.10.024

Norberg, B., Bjo¨rnsson, B. T., Brown, C. L., Wichardt, U.-P., Deftos, L. J., & Haux, C. (1989). Changes in plasma vitellogenin, sex steroids, calcitonin and thyroid hormones related to sexual maturation in female brown trout (*Salmo trutta*). *General and Comparative Endocrinology*, *75*, 316–326. https://doi.org/10.1016/0016-6480(89)90085-3

Norberg, B., Brown, C. L., Halldorsson, O., Stensland, K., & Bjørnsson, B. T. (2004). Photoperiod regulates the timing of sexual maturation,

spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). *Aquaculture*, *229*, 451–467. https://doi.org/10.1016/S0044-8486(03)00393-4

Önder, M. Y., Başçınar, N., Khan, U., & Sonay, F. D. (2016). Effect of Photoperiod on Growth and Efficiency of Yolk-Sac Utilization in Alevins of Brook Trout (*Salvelinus fontinalis*). *Pakistan Journal of Zoology*, *48*, 533–537.

Oppedal, F., Taranger, G. L., Juell, J. E., Fosseidengen, E., & Hansen, T. (1997). Light intensity affects growth and sexual maturation of Atlantic salmon (*Salmo salar*) postmolts in sea cages. *Aquatic Living Resources*, *10*, 351–357.

Pavlidis, M., Dimitriou, D., & Dessypris, A. (1994). Testosterone and 17-b-estradiol plasma fluctuations throughout spawning period in male and female rainbow trout, *Oncorhynchus mykiss* (Walbaum), kept under several photoperiod regimes. *Annales Zoologici Fennici*, *31*, 319–327.

Pickering, D. A., & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *Journal of Fish Biology*, *23*, 163–175. https://doi.org/10.1111/j.1095-8649.1983.tb02891.x

Rasmussen, R. S., & Ostenfeld, T. H. (2000). Effect of growth rate on quality traits and feed utilisation of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). *Aquaculture*, *184*, 327–337. https://doi.org/10.1016/S0044-8486(99)00324-5

Sacobie, C. F. D., Burke, H. A., Lall, S. P., & Benfley, T. J. (2016). The effect of dietary energy level on growth and nutrient utilization by juvenile diploid and triploid brook char, *Salvelinus fontinalis*. *Aquaculture Nutrition*, *22*, 1091–1100.

Saunders, R. L., Henderson, E. B., & Harmon, P. R. (1985). Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages. *Aquaculture*, *45*, 55–66. https://doi.org/10.1016/0044-8486(85)90257-1

Schulz, R. W., de França, L. R., Lareyre, J. J., LeGac, F., Chiarini Garcia, H., Nobrega, R. H., & Miura, T. (2010). Spermatogenesis in fish. *General and Comparative Endocrinology*, *165*, 390–411. https://doi.org/10.1016/j.ygcen.2009.02.013

Silversand, C., Hyllner, S. J., & Haux, C. (1993). Isolation, immunochemical detection, and observations of the instability of vitellogenin from four teleosts. *Journal of Experimental Zoology*, *267*, 587–597. https://doi.org/10.1002/jez.1402670606

Skjæraasen, J. E., Salvanes, A. G. V., Karlsen, Ø., Dahle, R., Nilsen, T., & Norberg, B. (2004). The effect of photoperiod on sexual maturation, appetite and growth in wild Atlantic cod (*Gadus morhua* L.). *Fish Physiology and Biochemistry*, *30*, 163–174. https://doi.org/10.1007/s10695-005-4319-6

Stejskal, V., Policar, T., Kristian, J., Kouril, J., & Hamackova, J. (2011). Fin condition in intensively cultured Eurasian perch (*Perca fluviatilis*). *Folia Zoologica*, *60*, 122–128.

Svinger, V., Policar, T., Steinbach, C., Polakova, S., Jankovych, A., & Kouril, J. (2013). Synchronization of ovulation in brook char (*Salvelinus fontinalis*, Mitchill 1814) using emulsified d-Arg6Pro9Net sGnRHa. *Aquaculture International*, *21*, 783–799. https://doi.org/10.1007/s10499-012-9578-5

Svobodova, Z., Kolarova, J., Navratil, S., Vesely, T., Chloupek, P., Tesarcik, J., & Citek, J. (2007). Nemoci sladkovodnich a akvarijnich ryb. Praha, Informatorium, 264 pp.

Tam, W. H., Roy, J. J., & Makaran, R. (1986). Ovarian cycle and plasma concentrations of estrogen and vitellogenin in brook trout (*Salvelinus fontinalis*). *Canadian Journal of Zoology*, *64*, 744–751.

Taranger, G. L., Aardal, L., Hansen, T., & Kjesbu, O. S. (2006). Continuous light delays sexual maturation and increases growth of Atlantic cod (*Gadus morhua* L.) in sea cages. *ICES Journal of Marine Science*, *63*, 365–375. https://doi.org/10.1016/j.icesjms.2005.10.014

Taranger, G. L., Haux, C., Hansen, T., Stefansson, S. O., Bjo¨rnsson, B. T., Walter, B. T., & Kryvi, H. (1999). Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. *Aquaculture*, *177*, 47–60. https://doi.org/10.1016/S0044-8486(99)00068-X

Taylor, J. F., Migaud, H., Porter, M. J. R., & Bromage, N. R. (2005). Photoperiod influences growth rate and insulin-like growth factor-I (IGF-I) levels in juvenile rainbow trout, *Oncorhynchus mykiss*. *General Comparative Endocrinology*, *142*, 169–185.

Urban, J. (2011/2012). Fish Skin Color Evaluation Application (FISCEAPP) [software]. Retrieved from https://www.auc.cz/software/index4.htm#FISCEAPP [1]

Urban, J. (2017). Colorimetric experiments on aquatic organisms. In F. Ortuno, & I. Rojas (Eds.), *IWBBIO*, *2017*, *Proceedings*, *Part I*, *LNBI 10208* (pp. 96–107). Switzerland: Springer.

Warren, D. R., Robinson, J. M., Josephson, D. C., Sheldon, D. R., & Kraft, C. E. (2012). Elevated summer temperatures delay spawning and reduce red construction for resident brook trout (*Salvelinus fontinalis*). *Global Change Biology*, *18*, 1804–1811.

Willoughby, L. G., & Pickering, A. D. (1997). Viable *Saprolegnia* spores on the epidermis of salmonid fish *Salmo trutta* and *Salvenilus alpinus*. *Transactions of the British Mycological Society*, *68*, 91–95.

Zajic, T., Mraz, J., Sampels, S., & Pickova, J. (2016). Finishing feeding strategy as an instrument for modification of fatty acid composition of brook trout (*Salvelinus fontinalis*). *Aquaculture International*, *24*, 1641–1656.

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CHAPTER 3

THE EFFECT OF TIMING OF EXTENDED PHOTOPERIOD ON GROWTH AND MATURITY OF BROOK TROUT (*SALVELINUS FONTINALIS*)

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The effect of timing of extended photoperiod on growth and maturity of brook trout (*Salvelinus fontinalis*)

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Abstract

The influence of timing of extended photoperiods on growth and maturity of brook trout was investigated in a 112-day experiment. The fish with mean initial weight of ~192 g were reared under four light regimes: one control group with natural ambient photoperiod and three groups exposed to an 18L:6D regime initiated at days 1, 23 or 46 of the growth trial. Light-emitting diodes, with intensity of 250–1000 lux, depending on the distance from the light source, were used for extending light periods. There was a positive effect of prolonged day length on fish growth ($p < 0.05$), and a delay in gonad development and sexual maturity. Significantly higher numbers of sexually mature fish were found among controls groups, regardless of sex. Survival rate was not affected by light regime. This study demonstrated that the short-term expansion of the photo period delayed maturation and increased the growth rate of brook trout.

KEYWORDS

growth, photoperiod, *Salvelinus fontinalis*, sexual maturation, timing

1 | INTRODUCTION

Development and maturation of fish are related to seasonal changes in weather, photoperiod and food availability. Maturation processes in fish, as well as other seasonally breeding animals, are regulated by ambient conditions via reception of signals from the external environment (Bromage, Porter, & Randall, 2001).

Sexual maturation is a problem in aquaculture (Taranger, Aardal, Hansen, & Kjesbu, 2006), especially in salmon farming (Migaud, Cowan, Taylor, & Ferguson, 2007). Sexual maturation in salmonids influences size, growth rate, energy reserves and the spawning period (Liu & Duston, 2016; McMillan, Dunham, Reeves, Mill, & Jordan, 2012). Onset of maturity occurs approximately 1 year before spawning and is regulated by the photoperiod. The course of the process is characterized by signs of gametogenesis, increased levels of sex steroids and sexual dimorphism in morphology (Andersson et al., 2013; Campbell et al., 2006). Onset of maturity leads to loss of body weight and increased aggression in males. This results in decreased resistance to disease and deterioration in flesh quality (Sacobie, Burke, Lall, & Benfley, 2016) and reduces

the proportion of edible flesh of total body weight. This negative impact manifested an increase in feed required to restore energy, which spent on gonad development, spawning and recovery, can jeopardize production efficiency and reduce the volume of commercially valuable biomass (Leclercq, Taylor, Spraque, & Migaud, 2011). Reduced growth during the spawning season increases the timing required to produce harvest-sized fish (Taranger et al., 2006). Control of pre-spawning sexual maturation is a priority in the salmon industry.

The annual photoperiod cycle is the main environmental zeitgeber influencing the reproductive cycle of salmonids (Taranger et al., 2010). Previous studies have demonstrated that the manipulation of photoperiod affects reproduction, growth rate and feeding behaviour in several species of salmonids without negative consequences (Boeuf & Le Bail, 1999; Brown, Baumann, & Conover, 2014). The primary mechanism underlying the delay of sexual maturation with photoperiod manipulation in salmonids remains unclear (Liu & Duston, 2016; Taranger et al., 2010), but it is suggested that the intensity and spectrum of light exposure affect the physiological responses of teleosts in areas such as growth, reproduction, behaviour and stress resistance (Boeuf & Le Bail, 1999; Karakatsouli et al., 2007; Migaud, Davie, & Taylor, 2010). The use of optimized light strategies can reduce operation costs and maximize growth rates with postponement of puberty (Leclercq et al., 2011).

Brook trout *Salvelinus fontinalis* is known as a relatively hardy and highly adaptable fish species for aquaculture systems (Jobling et al., 2010). Previous investigations have reported that brook trout is becoming the second most popular salmonid in Central Europe aquaculture (Svinger et al., 2013), but lack of knowledge in optimal technologies, especially for early maturation inhibition limits future growth in commercial culture in this area (Önder, Başçınar, Khan, & Sonay, 2016).

The primary aim of this study was to evaluate the effect of prolonging the photoperiod on sexual maturation, growth rate and overall performance of brook trout *S. fontinalis* and to determine the most effective timing of the prolonged photoperiod.

2 | MATERIALS AND METHODS

2.1 | Experimental protocol

Randomly collected *S. fontinalis* (n = 1,800) of the same age category with an average mean weight of ~192 ± 37.9 g were distributed among 12 outdoor plastic rectangular flow-through tanks (2.7 m^3, 300 × 100 × 90 cm) with water flow rate of 3–3.5 m^3 h^{-1}. Fish were divided into four groups of 450 individuals (150 per tank, three replicates of each treatment). All tanks were equipped with an aeration system and a lighting system using light-emitting diode (LED) lights. Two light sources consisting of an Epistar 50 W LED CO8 (50 W, 4,000 Lm, 6,000 K) with a set intensity of 250–1000 lux, depending on the distance from the light source, were placed above each tank. The height of light sources from the water level was 50 cm.

Four groups of fish under different timing of photoperiod regimes were tested. The beginning of the experiment was on day 46 post-midsummer (46 DPM). Group 46 DPM was exposed to an extended photoperiod (18L:6D) beginning from 6 August 2015 (day 46 post-midsummer). Group 67 DPM was exposed to an extended photoperiod (18L:6D) starting from 27 August (day 67 post-midsummer). Group 88 DPM received the extended photoperiod (18L:6D) starting from 17 September (day 88 post-midsummer). A control group (CON) was reared under natural ambient light conditions (Figure 1).

Fish were fed Biomar EFICO Alpha 756 (protein 39%–42%, fat 21%–24%, carbohydrates 21%, fibre 4.7%, ash 5.9%, total phosphorus 0.8%, gross energy 20–24 MJ/kg, digestible energy 18.7 MJ/kg) twice per day at rates recommended by the manufacturer, from 08:00 to 15:00 hours. The fish were not fed for 48 hr before data collection. Monitoring of dissolved oxygen, temperature, pH and fish mortality was carried out twice per day at 08:00 and 15:00 hours. Light intensity was measured by a Hydroluxunder water illumination meter (lux meter) (BGB Innovation).

2.2 | Data sampling and analysis

The experiment was carried out from 5 August 2016 to 24 November 2016 (112 days). On days 28, 56, 84 and 112 of the

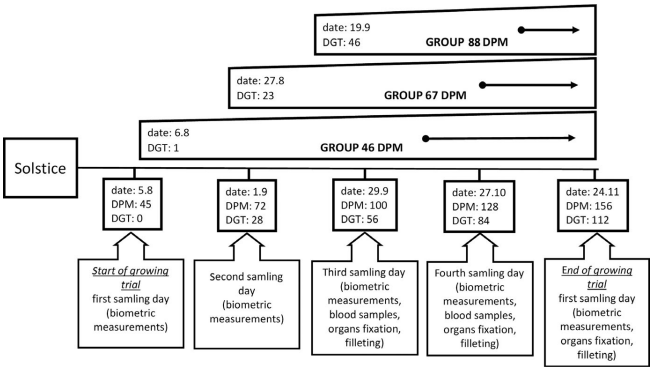

FIGURE 1 Experimental design. DPM = days post-midsummer (midsummer = summer solstice). DGT = days of growth trial

growth trial, fish were anaesthetized with clove oil (0.03 ml/L), and body weight (BW) and total length (L_T) of 50 fish from each experimental tank (n = 150 per group) were measured. On 56, 84 and 112 days of the growth trial, 30 males and 30 females from each experimental treatment were killed with an overdose of anaesthetic, dissected and gonads were removed and fixed in Bouin's solution for the calculation of:

$$\text{Condition factor } (K) = 100 \times BW \times T_L^{-3};$$

$$\text{Hepatosomatic index } (HSI) = 100 \times \text{liver weight}/BW;$$

$$\text{Gonadosomatic index } (GSI) = 100 \times \text{gonad weight}/BW;$$

$$\text{Perivisceral fat index } (PVSI) = 100 \times \text{fat weight}/BW;$$

$$\text{Fillet yield } (FY) = 100/BW \times \text{fillets weight}.$$

Thirty males and 30 females from each group were used for blood sampling on days 56, 84 and 112 of the growth trial. Plasma glucose level was analysed on an Architect 8000 chemistry analyser (Abbott Laboratories) using the hexokinase/G-6-PDH spectrophotometric assay. Oestradiol and testosterone levels were determined by solid-phase, enzyme-labelled chemiluminescent competitive immunoassays on the Immulite 2000 XPi immunoassay system (Siemens). Plasma sample volumes for analyses were 2 µl for glucose, 25 µl for oestradiol and 20 µl for testosterone.

2.3 | Statistical analysis

Data were analysed with Microsoft Excel 2010 (Microsoft, Inc.). Statistical analysis consisted of a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (Statistica 12.0; StatSoft, Inc.). When the ANOVA assumptions were not satisfied, the differences between groups were tested using the nonparametric Kruskal–Wallis test. The level of significance for all analyses was $p < 0.05$.

3 | RESULTS

The fish reared under the different photoperiod regimes differed in growth patterns. Figures 2 and 3 present changes in BW in female and male brook trout respectively. Body weight increased in both sexes until day 84 of the growth trial. From this time until the end of the experiment, weight loss was recorded in controls, regardless of sex. Fish groups reared under the prolonged photoperiod showed significantly higher mean BW and total length (T_L) compared to controls ($p < 0.05$) (Figures 2 and 3). At the end of study trial, significantly lower T_L was found in the control groups (270 ± 21.4 mm – females and 284 ± 20.38 mm – males).

There were no significant differences among groups in mean K throughout the trial, except at the end of the experiment, the controls showed significantly lower K (1.08 ± 0.1 in females and 1.22 ± 0.16 in males) compared to other treatments, regardless of sex ($p < 0.05$) (Figures 4 and 5).

The gonadosomatic index (GSI) in the controls and in the 88 DPM group was similar, regardless of sex (Figures 4 and 5). At day 84 of the growth trial, significantly higher mean GSI was observed in the female control group compared to other treatments ($p < 0.05$) (Figure 4). In the same time period, the male controls showed significantly lower GSI compared to experimental treatments (Figure 5).

No significant changes were found in male hepatosomatic index (HSI) during the experimental period (Figure 5). The opposite situation was observed in females: At days 56 and 84 of the growth trial, the female controls showed significantly higher mean HSI compared with other treatments ($p < 0.05$). And at the end of trial, the highest HSI was observed in the 67 DPM and 46 DPM groups ($p < 0.05$) (Figure 4).

Significantly lower perivisceral fat index (PVSI) was found in control females compared to other treatments on days 84 and 112 (1.61 ± 0.7 and 1.17 ± 0.44 respectively) (Figure 4). At the start of the trial, 46 DPM males showed significantly higher PVSI than all other groups ($p < 0.05$). On day 84 of the growth trial, control male sex habited significantly lower PVSI (2.0 ± 0.59) compared with 67 DPM and 46 DPM (Figure 5).

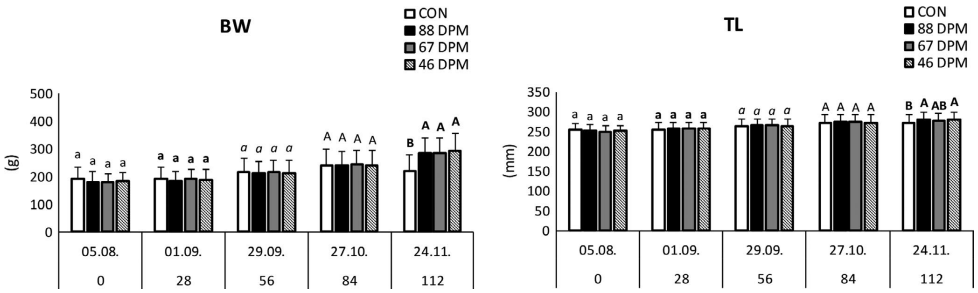

FIGURE 2 Female brook trout body weight (BW, g) and total length (TL, mm) exposed to different photoperiods (n = 150). Data are shown as mean (bars) ± *SD* (whiskers). Different letters above bars indicate significant difference among treatments at a sampling date ($p < 0.05$). Various types of letters (capital, italics, etc.) indicate different sampling dates

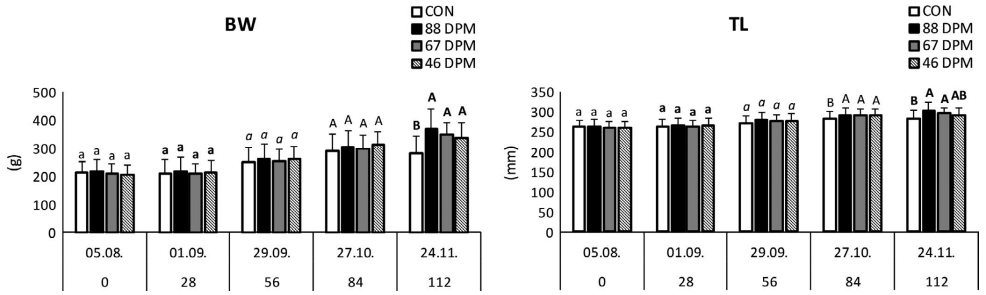

FIGURE 3 Male brook trout body weight (BW, g) and total length (TL, mm) exposed to different photoperiods (n = 150). Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$).Various types of letters (capital, italics, etc.) indicate different sampling dates

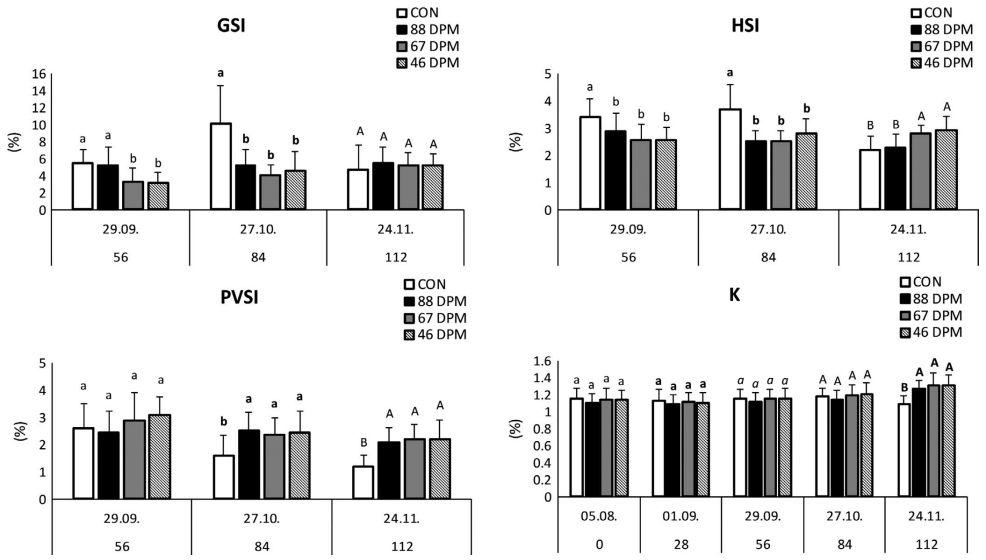

FIGURE 4 Gonadosomatic index (GSI, %), hepatosomatic index (HSI, %), perivisceral fat index (PVSI, %) and condition factor (K) of female brook trout reared under different photoperiods. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$). Various types of letters (capital, italics, etc.) indicate different sampling dates

Both sexes presented significant differences in fillet yield (FY) during the study. On day 56 of the growth trial, lowest FY was observed in controls and 88 DPM, regardless of sex ($p < 0.05$). The control females showed significantly lower FY compared to experimental treatments on day 84 (Figure 6).

The lowest levels of glucose were observed in control females ($p < 0.05$). At the end of the trial, significantly higher glucose was found in 46 DPM males compared to controls (Figure 7). Among male groups, significantly higher testosterone was observed in controls on days 56 and 84 of the growth trial. On days 56 and 84 of the growth trial, control females showed highest testosterone level among female groups ($p < 0.05$). On day 112, the opposite situation was revealed, and female control showed lowest testosterone level (Figure 7). A similar pattern was observed in oestradiol content among female groups (Figure 7). Significantly higher oestradiol was found in male 88 DPM on day 56 than seen in other male groups. On day 84 of the growth trial, control males exhibited significantly higher oestradiol compared to the 88 DPM and 46 DPM treatments ($p < 0.05$). And at the end of the experiment, the 88 DPM males showed significantly higher oestradiol levels than observed in the controls ($p < 0.05$) (Figure 7).

At the end of the trial, the fish were evaluated for development of gonads and secretion of sperm and eggs by manual expression

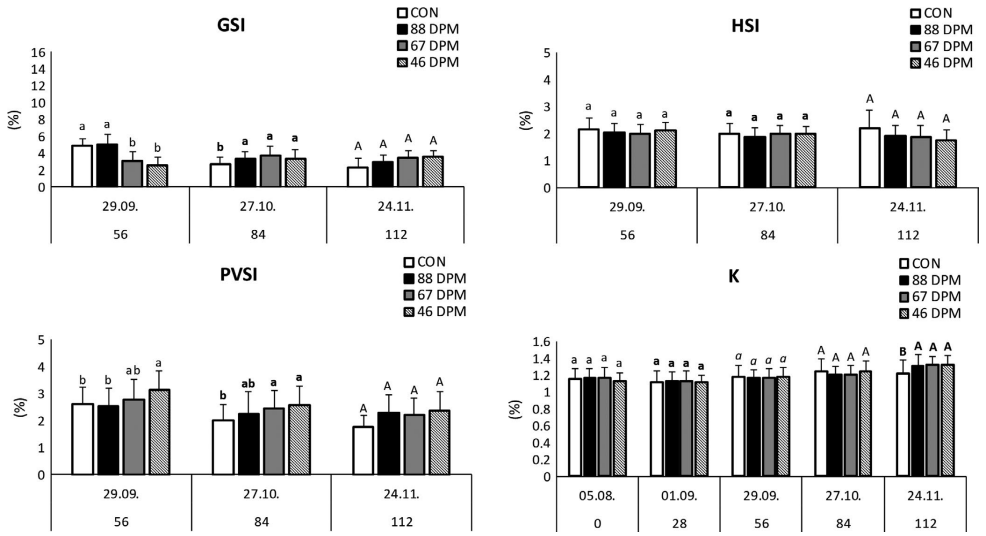

FIGURE 5 Gonadosomatic index (GSI, %), hepatosomatic index (HSI, %), perivisceral fat index (PVSI, %) and condition factor (K) of male brook trout reared under different photoperiods. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$). Various types of letters (capital, italics, etc.) indicate different sampling dates

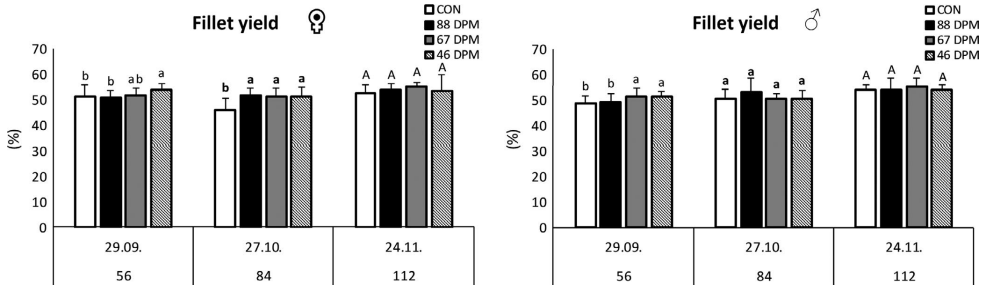

FIGURE 6 Fillet yield (%) of brook trout reared under different photoperiods. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$). Various types of letters (capital, italics, etc.) indicate different sampling dates

of gametes. High percentages of fish from the control groups were mature. A lower, but non-significant, number of males from 46 DPM, 67 DPM and 88 DPM groups exhibited spawning readiness. No females exposed to the prolonged photoperiod were ready to spawn (Figure 8).

Similar mortality occurred in all experimental treatments. A higher number of dead fish were observed among controls from days 105 to 109 compared to the other groups (Figure 9). At the conclusion of this study, there were no significant differences in survival rate among treatments (control = 68.2 ± 3.1%, 88 DPM = 77.1 ± 9.8%, 46 DPM = 71.8 ± 15.6%). The highest mean survival was seen in the 67 DPM group (81.3 ± 10.1%).

At the end of experiment, the energy cost of providing the extended photoperiod was calculated. The cost of artificial prolongation of daylight was 93€ for 46 DPM, 75€ for 67 DPM and 56€ for 88 DPM, making the 88 DPM treatment the most cost effective.

4 | DISCUSSION

Results of the study demonstrated that using an artificially prolonged photoperiod improved growth and retarded the sexual maturation of brook trout. Changes in growth rate are consistent with earlier studies showing that continuous artificial light has a growth-promoting

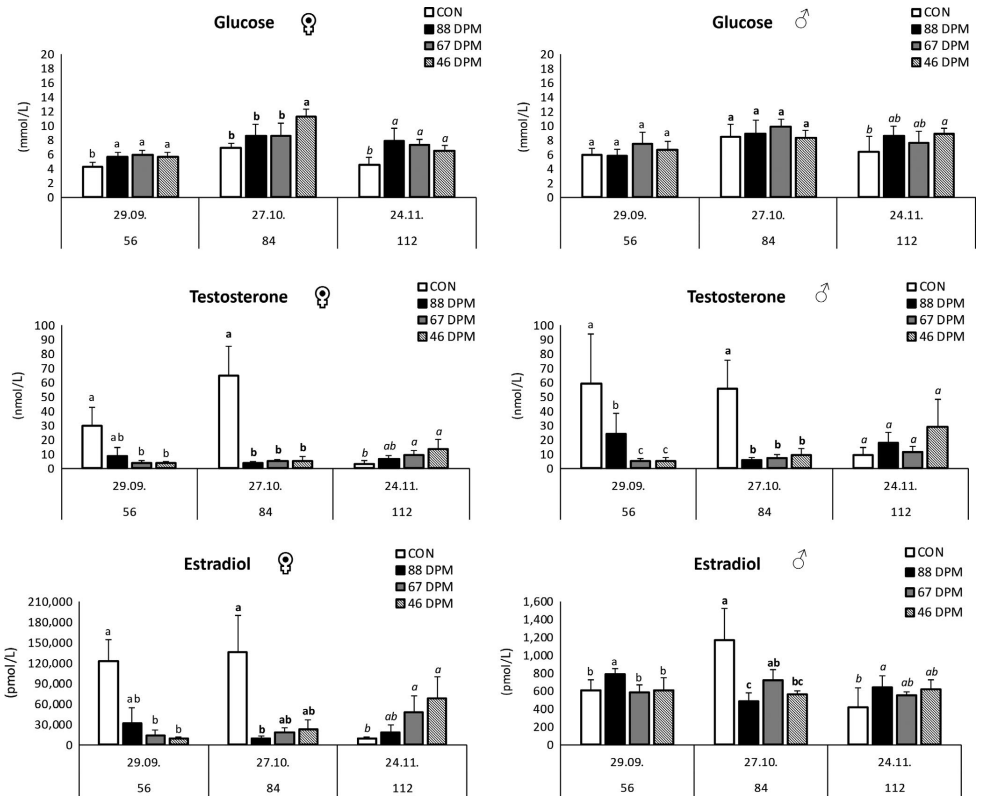

FIGURE 7 Temporal changes in glucose, testosterone and oestradiol concentrations in blood plasma of female and male brook trout under different light regimes. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$). Various types of letters (capital, italics, etc.) indicate different sampling dates

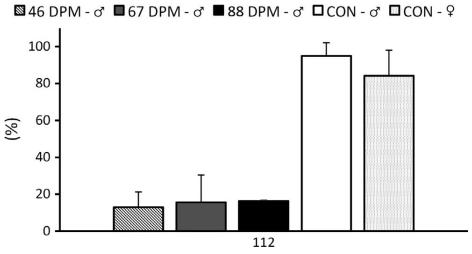

FIGURE 8 Spawning readiness of brook trout reared under different light regimes. Data are shown as mean (bars) ± *SD* (whiskers)

effect on salmonids (Hansen, Fjelldal, Folkedal, Vagseth, & Oppedal, 2017; Önder et al., 2016; Taylor, Migaud, Porter, & Bromage, 2005). The growth stimulation has not been shown affected by an increase in the feeding period, but by increase of daylight hours per se (Taylor, North, Porter, Bromage, & Migaud, 2006). Randall, North, Futter, Porter, and Bromage (2001) also reported growth-enhancing effects of continuous artificial lighting in rainbow trout without additional feed. At the end of this study, all fish exposed to an 18L:6D photoperiod had higher body weight compared to the fish reared under ambient light conditions. This was confirmed by a similar pattern in total body length and condition factor. The effect of the duration of artificial photoperiod on the growth characteristics can be explained by reallocation of energy from gonad development to somatic growth with delay of puberty (Gines, Afonso, Arguello, Zamorano, & Lopez, 2003; Noori, Amiri, Mirvaghefi, Rafiee, & Neitali, 2015). This statement is clearly supported by the GSI indicators in the female groups. The female controls showed significantly higher GSI during the natural pawning time for brook trout. Over time, the GSI of the groups exposed to the prolonged photoperiod began to increase, and by the end of the study the indices were similar. After the

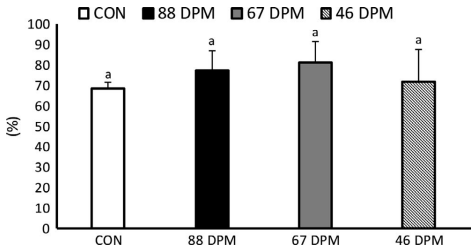

FIGURE 9 Final survival rate of brook trout reared under different photoperiods. Data are shown as mean (bars) ± *SD* (whiskers). Different letters indicate significant difference among treatments at a sampling date ($p < 0.05$)

conclusion of the trial, fish from the experimental groups reached puberty. A similar trend was observed in the males. These results support the assumption that the onset of sexual maturity in the fish was delayed by artificially prolonged daylight.

Sex steroids play a key role in fish gametogenesis, in accumulating vitellogenin in the oocytes (Silversand, Hyllner, & Haux, 1993) and the production of spermatogonia during spermatogenesis (Schulz et al., 2010). Kagawa, Young, and Nagahama (1983) reported that plasma sex steroids show a linear correlation with GSI in female salmon. The data obtained in this study confirmed this statement: a significant increase of oestradiol in the female controls compared to the other treatments was observed. A subsequent sharp decrease in this indicator was noted at the end of experiment in the control group. These data are in agreement with studies of other salmonids, in which the level of oestradiol returns to baseline concentrations prior to the onset of ovulation (Frantzen, Arnesen, Damsgård, Tveiten, & Johnsen, 2004; Pavlidis, Dimitriou, & Dessypris, 1994). The detected elevated testosterone level in control females can be explained by the fact that it is a precursor for oestrogens and positively affects gonadotropin production (Dickhoff & Swanson, 1990; Schulz & Miura, 2002).A similar situation was observed in control males. The data obtained on testosterone levels in males are in agreement with that reported by Holcombe, Pasha, Jensen, Tietge, and Ankley (2000) for brook trout and Qiu et al. (2015) for Atlantic salmon.

These data are also confirmed by the results observed in controls with respect to readiness for spawning at the end of trial. The majority of control males (95%) and 84% of control females had matured. Approximately 15% of males of the experimental treatments were spawning-ready, but we observed no sexually mature females.

The light regimes showed no significant effect on the survival rates of brook trout reared under prolonged photoperiods. Similar results were obtained by Qiu et al. (2015) who evaluated the influence of spectral composition, photoperiod and light intensity on Atlantic salmon *Salmo salar*. In our study, the lowest mean survival rate (68.2%) was seen in fish reared under natural ambient light. In further studies, we propose to pay more attention to this issue and to conduct thorough experiments on the effect of an artificially prolonged photoperiod on the mortality rate of salmonid fish species.

This study demonstrated that artificial prolongation of daylight can improve the growth rate and delay sexual maturation of brook trout. Based on the obtained results, we can recommend the use of a 66-day extended photoperiod from 19 September to 24 November, since we found no differences in growth parameters and maturation among the groups exposed to the prolonged daylight regimes, and the shorter duration of artificial lighting was the least costly. Short-term application of the artificial prolonged photoperiod can be effective in suppressing and delaying gonad development and increasing the growth rate of brook trout.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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REFERENCES

Andersson, E., Schulz, R. W., Male, R., Bogerd, J., Patiña, D., Benedet, S., … Taranger, G. L. (2013). Pituitary gonadotropin and ovarian gonadotropin receptor transcript levels: Seasonal and photoperiod-induced changes in the reproductive physiology of female Atlantic salmon (*Salmo salar*). *General and Comparative Endocrinology*, *191*, 247–258. https://doi.org/10.1016/j.ygcen.2013.07.001

Boeuf, G., & Le Bail, P. Y. (1999). Does light have an influence on fish growth? *Aquaculture*, *177*, 129–152.

Bromage, N., Porter, M., & Randall, C. (2001). The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture*, *197*, 63–98. https://doi.org/10.1016/S0044-8486(01)00583-X

Brown, E. E., Baumann, H., & Conover, D. O. (2014). Temperature and photoperiod effects on sex determination in a fish. *Journal of Experimental Marine Biology and Ecology*, *461*, 39–43. https://doi.org/10.1016/j.jembe.2014.07.009

Campbell, B., Dickey, J., Beckman, B., Young, G., Pierce, A., Fukada, H., & Swanson, P. (2006). Previtellogenic oocyte growth in salmon: Relationships among body growth, plasma insulin-like growth factor-1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic-acute regulatory protein and receptors for gonadotropins, growth hormone and somatolactin. *Biology of Reproduction*, *75*, 34–44.

Dickhoff, W. W., & Swanson, P. (1990). Function of salmon pituitary glycoprotein hormones: "The maturational surge hypothesis". *Progress in Clinical and Biological Research*, *342*, 349–356.

Frantzen, M., Arnesen, A. M., Damsgård, B., Tveiten, H., & Johnsen, H. K. (2004). Effects of photoperiod on sex steroids and gonad maturation in Arctic charr. *Aquaculture*, *240*, 561–574. https://doi.org/10.1016/j.aquaculture.2004.07.013

Gines, R., Afonso, J. M., Arguello, A., Zamorano, M. J., & Lopez, J. L. (2003). Growth in adult gilthead sea bream (*Sparus aurata* L.) as a result of interference in sexual maturation by different photoperiod regimes. *Aquaculture Research*, *34*, 73–83. https://doi.org/10.1046/j.1365-2109.2003.00801.x

Hansen, T. J., Fjelldal, P. G., Folkedal, O., Vagseth, T., & Oppedal, F. (2017). Effects of light source and intensity on sexual maturation, growth and swimming behaviour of Atlantic salmon in sea cages. *Aquaculture Environment Interactions*, *9*, 193–204. https://doi.org/10.3354/aei00224

Holcombe, G. W., Pasha, M. S., Jensen, K. M., Tietge, J. F., & Ankley, G. T. (2000). Effects of photoperiod manipulation on brook trout reproductive development, fecundity, and circulating sex steroid concentrations. *North American Journal of Aquaculture*, *62*, 1–11. https://doi.org/10.1577/1548-8454(2000)062<0001:EOPMOB>2.0.CO;2

Jobling, M., Arnesen, A. M., Benfey, T. J., Carter, C. G., Hardy, R. W., LeFrancois, N. R., & Lamarre, S. G. (2010). The salmonids. In N. R. LeFrancois (Ed.), *Finfish aquaculture diversification* (pp. 234–289). Wallingford, UK: CAB International.

Kagawa, H., Young, G., & Nagahama, Y. (1983). Relationship between seasonal plasma estradiol-17 beta and testosterone levels and in vitro production by ovarian follicles of amago salmon (*Oncorhynchus rhodurus*). *Biology of Reproduction*, *29*, 301–309.

Karakatsouli, N., Papoutsoglou, S. E., Pizzonia, G., Tsatsos, G., Tsopelakos, A., Chadio, S., … Papadopoulou-Daifoti, Z. (2007). Effects of light spectrum on growth and physiological status of gilthead sea bream *Sparus aurata* and rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions. *Aquacultural Engineering*, *36*, 302–309. https://doi.org/10.1016/j.aquaeng.2007.01.005

Leclercq, E., Taylor, J. F., Spraque, M., & Migaud, H. (2011). The potential of alternative lighting-systems to suppress pre-harvest sexual maturation of 1+ Atlantic salmon (*Salmo salar*) post-smolts reared in commercial sea-cages. *Aquacultural Engineering*, *44*, 35–47. https://doi.org/10.1016/j.aquaeng.2010.12.001

Liu, Q., & Duston, J. (2016). Preventing sexual maturation in Arctic charr by 24 h light overwinter and suppressing somatic growth. *Aquaculture*, *464*, 537–544. https://doi.org/10.1016/j.aquaculture.2016.07.038

McMillan, J. R., Dunham, J. B., Reeves, G. H., Mill, J. S., & Jordan, C. E. (2012). Individual condition and stream temperature influence early maturation of rainbow trout and steelhead trout, *Oncorhynchus mykiss*. *Environmental Biology of Fishes*, *93*, 343–355.

Migaud, H., Cowan, M., Taylor, J., & Ferguson, H. W. (2007). The effect of spectral composition and light intensity on melatonin, stress and retinal damage in post-smolt Atlantic salmon, *Salmo salar*. *Aquaculture*, *270*, 390–404. https://doi.org/10.1016/j.aquaculture.2007.04.064

Migaud, H., Davie, A., & Taylor, J. F. (2010). Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. *Journal of Fish Biology*, *76*, 27–68. https://doi.org/10.1111/j.1095-8649.2009.02500.x

Noori, A., Amiri, B. M., Mirvaghefi, A., Rafiee, G., & Neitali, B. K. (2015). Enhanced growth and retarded gonadal development of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum) following a long-day photoperiod. *Aquaculture Research*, *46*, 2398–2406.

Önder, M. Y., Başçınar, N., Khan, U., & Sonay, F. D. (2016). Effect of photoperiod on growth and efficiency of yolk-sac utilization in alevins of brook trout (*Salvelinus fontinalis*). *Pakistan Journal of Zoology*, *48*, 533–537.

Pavlidis, M., Dimitriou, D., & Dessypris, A. (1994). Testosterone and 17-b-estradiol plasma fluctuations throughout spawning period in male and female rainbow trout, *Oncorhynchus mykiss* (Walbaum), kept under several photoperiod regimes. *Annales ZoologiciFennici*, *31*, 319–327.

Qiu, D., Xu, S., Song, C., Chi, L., Li, X., Sun, G., … Liu, Y. (2015). Effects of spectral composition, photoperiod and light intensity on the gonadal development of Atlantic salmon *Salmo salar* in recirculating aquaculture systems (RAS). *Chinese Journal of Oceanology and Limnology*, *33*, 45–56. https://doi.org/10.1007/s00343-015-4011-3

Randall, C., North, B., Futter, W., Porter, M., & Bromage, N. (2001). Photoperiod effects on reproduction and growth in rainbow trout. *Trout News*, *32*, 12–16.

Sacobie, C. F. D., Burke, H. A., Lall, S. P., & Benfley, T. J. (2016). The effect of dietary energy level on growth and nutrient utilization by juvenile diploid and triploid brook char, *Salvelinus fontinalis*. *Aquaculture Nutrition*, *22*, 1091–1100.

Schulz, R. W., de Franca, L. R., Lareyre, J. J., LeGac, F., Chriarini Garcia, H., Nobrega, R. H., & Miura, T. (2010). Spermatogenesis if fish. *General and Comparative Endrocrinology*, *165*, 390–411.

Schulz, R. W., & Miura, T. (2002). Spermatogenesis and its endocrine regulation. *Fish Physiology and Biochemistry*, *26*, 43–56.

Silversand, C., Hyllner, S. J., & Haux, C. (1993). Isolation, immunochemical detection, and observations of the instability of vitellogenin from four teleosts. *Journal of Experimental Zoology*, *267*, 587–597. https://doi.org/10.1002/jez.1402670606

Svinger, V., Policar, T., Steinbach, C., Polakova, S., Jankovych, A., & Kouril, J. (2013). Synchronization of ovulation in brook char (*Salvelinus fontinalis*, Mitchill 1814) using emulsified d-Arg_6Pro_9Net sGnRHa. *Aquaculture International*, *21*, 783–799.

Taranger, G. L., Aardal, L., Hansen, T., & Kjesbu, O. S. (2006). Continuous light delays sexual maturation and increases growth of Atlantic cod (*Gadus morhua* L.) in sea cages. *ICES Journal of Marine Science*, *63*, 365–375. https://doi.org/10.1016/j.icesjms.2005.10.014

Taranger, G. L., Carrillo, M., Schulz, R. W., Fontaine, P., Zanuy, S., Felip, A., … Hansen, T. (2010). Control of puberty in farmed fish. *General and Comparative Endocrinology*, *165*, 483–515. https://doi.org/10.1016/j.ygcen.2009.05.004

Taylor, J. F., Migaud, H., Porter, M. J. R., & Bromage, N. R. (2005). Photoperiod influences growth rate and insulin-like growth factor-I (IGF-I) levels in juvenile rainbow trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology*, *142*, 169–185.

Taylor, J. F., North, B. P., Porter, M. J. R., Bromage, N. R., & Migaud, H. (2006). Photoperiod can be used to enhance growth rate and improve feeding efficiency in farmed rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *256*, 216–234.

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CHAPTER 4

THE EFFECT OF NON-CIRCADIAN PHOTOPERIOD ON GROWTH AND PUBERTY ONSET OF BROOK TROUT *SALVELINUS FONTINALIS* MITCHILL

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My share on this work was about 50%.

The effect of non-circadian photoperiod on growth and puberty onset of brook trout *Salvelinus fontinalis* Mitchill

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Abstract

The effect of a prolonged photoperiod on growth rate and sexual maturation in brook trout *Salvelinus fontinalis* was determined over 140 days (21 June to 07 November) under three photoperiod regimes in which fish were exposed to 24 h continuous light alternating with 24 or 48 h under the ambient photoperiod or 48 h continuous light alternating with 24 h ambient photoperiod. A control group was reared under the natural ambient photoperiod. Four-hundred-fifty fish with average initial body weight 101.3 ± 1.2 g were used for each experimental group (three replicates of each treatment plus control). The experimental group with 24 h continuous light/48 h ambient showed delayed gonad development and onset of puberty and increased somatic growth in both sexes. A significantly higher percentage of sexually mature fish was observed in the control group. We found significantly fewer sexually mature females compared to males. We recommend a regimen under which fish are exposed to 48 h of natural ambient light photoperiod alternating with 24 h of constant light.

Keywords: *Salvelinus fontinalis, photoperiod, growth, sexual maturation, puberty*

Introduction

Brook trout *Salvelinus fontinalis* Mitchill is a North American salmonid that has become popular in central and northern European aquaculture in recent decades (Svinger et al., 2013). It is highly adaptable to a range of aquaculture systems, tolerant to low pH and a wide range of temperature, and produces highly palatable meat (Zajic et al., 2016; Lundova et al., 2019). As with most salmonids, the typical spawning period is autumn (Holcombe et al., 2000), and photoperiod is an important factor regulating synchronous spawning (Skjæraasen et al., 2004).

Despite the success in developing rearing technologies and the growing market interest, early sexual maturation is a major problem in the on-growing industry. Precocious maturation has a negative impact on growth rate, the immune system, and product quality (Harris and Bird 2000; Fjelldal et al., 2012; Stien et al., 2013; Linhartova et al., 2018; Lundova et al., 2019). In early puberty, fish show high growth potential, a factor increasing profitability in the farming industry (Iversen et al., 2016), but somatic weight subsequently stabilizes or decreases, as resources are diverted to gonad formation and gamete production (Taranger et al., 2010).

Sexual maturation of salmonids is controlled by factors including body size, growth rate, and fat deposition (Jonsson et al., 2012), as well as abiotic influences such as temperature, photoperiod, diet, stress, and behaviour (Thorpe et al., 1998; Pankhurst and Munday, 2011). In vertebrates, photoperiod has a significant effect on the activity of the brain pituitary-adrenal axis and, as a result, on gonad maturation (Bromage et al., 1993). Photoperiod manipulation can affect reproduction, growth rate, and feeding of salmonids without negative

outcome (Brown et al., 2014). Thus, photoperiod manipulation is used in fish farming to increase efficiency and enable year-round reproduction (Türker et al., 2011; Önder et al., 2016). Sexual maturation presents a number of problems that threaten the stability of aquaculture production (Schulz et al., 2006).

We hypothesize that application of a non-circadian photoperiod will influence the onset of puberty and result in reallocation of energy before and during the spawning period. The aim of this study was to determine the effect of a non-circadian photoperiod regimen on growth performance and puberty of brook trout.

Material and methods

Experimental protocol

The study was carried out from 21 June to 7 November (140 days) at Rybářství Litomyšl Ltd, Czech Republic.

Randomly collected *S. fontinalis* (n = 1800) mean weight 101.3 ± 1.2 g were distributed among twelve outdoor aerated flow-through tanks (water depth 0.85 m, volume 2.5 m^3) with flow rate of 3.0–3.5 $m^3.h^{-1}$. Fish were separated into four groups consisting of 150 specimens in each of three tanks (450 fish per group, three replicates of each treatment). Three treatment tanks were equipped with a system of light-emitting diodes (LED). Two lights, each fitted with an Epistar 50 W LED CO8 (50 W, 4000 Lm, 6000 K), were placed 50 cm above the water level in each tank, producing light intensity of 250–1000 lux as measured with a Hydrolux underwater illumination meter (luxmeter) (BGB Innovation) from c. 0.4 m below water surface. Three groups of fish were exposed to non-circadian photoperiod regimes. Group 1CP:1AP was exposed to 24 h of ambient photoperiod alternating with a 24 h of continuous light for 140 days (70 days of continuous light in total). Group 2CP:1AP was exposed to 24 h of ambient photoperiod alternating with 48 h continuous light (94 days of continuous light in total). Group 1CP:2AP were exposed to 48 h of natural ambient photoperiod alternating with 24 h of continuous photoperiod (47 days of continuous light in total). A fourth group (CON) was reared throughout 140 days under the natural ambient photoperiod as a control (Figure 1). Fish were manually fed twice (08:00 and 15:00) a day with fed Biomar EFICO Alpha 756 (protein 39–42%, fat 21–24%, carbohydrate 21%). The daily ration was adjusted to water temperature in accordance with the manufacturer's recommendations. Fish were unfed for 48 h prior to data collection. Dissolved oxygen, temperature, pH, and fish mortality were recorded twice daily. The pH value during the experiment ranged from 7.8 to 8.2, dissolved oxygen content was 100–110%.

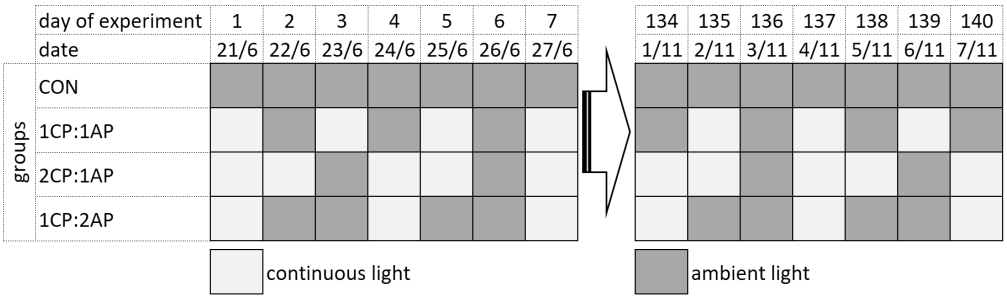

Figure 1. *Experimental design for testing non-circadian photoperiod on growth and puberty onset of brook trout Salvelinus fontinalis.*

Data collection and sample analysis

On days 28, 56, 84, 112, and 140, 30 females and 30 males from each tank (n = 90 females and n = 90 males per group) were anaesthetized with clove oil (0.03 $mL.L^{-1}$) for body weight (BW) and total length (L_T) measurement. On days 84, 112, and 140 30 males and 30 females (10 per tank) from each treatment plus control were killed with an over-dose of anaesthetic. Internal organs and gonads were removed and fixed in Bouin's solution for calculation of:

Hepatosomatic index (HSI) = 100 × liver weight/BW;

Gonadosomatic index (GSI) = 100 × gonad weight/BW;

Perivisceral fat index (PVSI) = 100 × fat weight/BW;

Condition factor (K) = $(BW/L^3) \times 100$;

Fillet yield (FY) = 100/BW × fillet weight.

Blood samples were collected from 30 males and 30 females from each group on days 84, 112, and 140. Plasma glucose was measured in an Architect 8000 biochemistry analyser (Abbott Laboratories, USA), and hormones in the Immulite 2000XPi immunoassay system (Siemens). Oestradiol and testosterone were measured by solid-phase, enzyme-labelled chemiluminescent competitive immunoassays, and glucose by spectrophotometric assay. Volume of plasma samples were 2 µL for glucose, 25 µL for oestradiol, and 20 µL for testosterone. Male and female fish data were analysed separately.

Statistical analysis

Data were analysed with Microsoft Excel 2010 (Microsoft, Inc., USA). Statistical analysis consisted of a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (Statistica 12.0; StatSoft, Inc., USA). When the ANOVA assumptions were not satisfied, the differences between groups were tested using the nonparametric Kruskal-Wallis test. During statistical analyzing a Bonferroni correction was applied to adjust the *p*-values. The level of significance for all analyses was $p < .05$.

Results

At the end of the growth experiment, the CON group showed significantly lower mean BW in both sexes females, 199.8 ± 12.2 g and males, 226.6 ± 39.8 g compared to the experimental groups (Figures 2 (females) and 3 (males)). The same was observed for L_T, with the CON group having lower mean length compared to all experimental groups ($p < .05$), regardless of sex (females, 258 ± 15 mm and males, 264 ± 14 mm (Figures 2, 3) (Table 1).

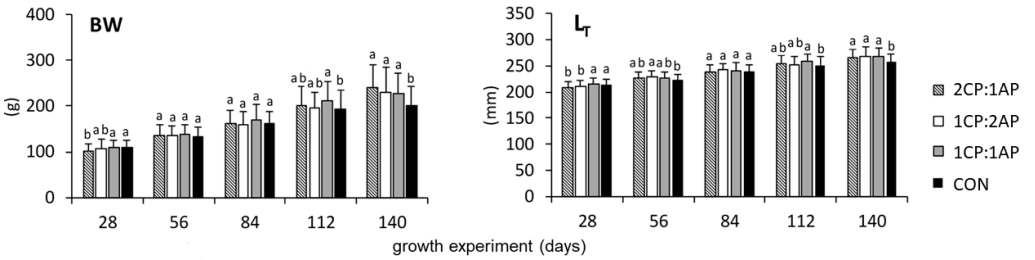

Figure 2. *Female brook trout body weight (BW, g), total length (L_T, mm) after exposure to three non-circadian photoperiods (see Figure 1 for details). Data are mean (bars) ± SD (whiskers). Different letters above bars indicate significant difference among treatments at a sampling date ($p < .05$).*

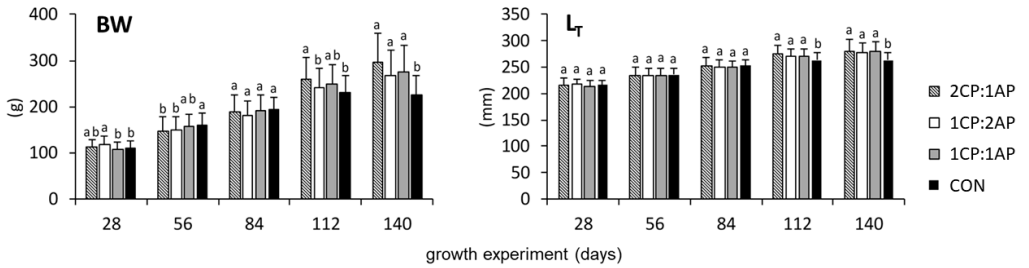

Figure 3. *Male brook trout body weight (BW, g), total length (L_T, mm) after exposure to three non-circadian photoperiods (see Figure 1 for details). Data are mean (bars) ± SD (whiskers). Different letters above bars indicate significant difference among treatments at a sampling date (p < .05).*

Table 1. *Summary of statistical model for brook trout body weight (BW), total length (L_T) after exposure to three non-circadian artificial photoperiods.*

Parameter	Sampling day	Test type	Chi-squared / F-value	df	p-value
		Females			
BW	28	ANOVA	4.292	3	**0.005**
	56	ANOVA	1.306	3	0.272
	84	Kruskal-Wallis	2.698	3	0.441
	112	Kruskal-Wallis	10.897	3	**0.012**
	140	Kruskal-Wallis	33.894	3	**< 0.001**
L_T	28	ANOVA	5.511	3	**0.001**
	56	Kruskal-Wallis	10.785	3	**0.013**
	84	ANOVA	0.784	3	0.504
	112	ANOVA	3.836	3	**0.01**
	140	ANOVA	8.123	3	**< 0.001**
		Males			
BW	28	ANOVA	4.675	3	**0.003**
	56	Kruskal-Wallis	12.563	3	**0.006**
	84	ANOVA	2.307	3	0.077
	112	ANOVA	6.412	3	**0.0003**
	140	Kruskal-Wallis	48.2	3	**< 0.001**
L_T	28	Kruskal-Wallis	6.08	3	0.108
	56	ANOVA	0.13	3	0.942
	84	Kruskal-Wallis	5.847	3	0.119
	112	Kruskal-Wallis	22.181	3	**< 0.001**
	140	Kruskal-Wallis	46.288	3	**< 0.001**

Note. Model types given as subheadings; see main text and Figure 2, 3 for details. df – degrees of freedom. Significant values (p < .05) in bold.

On day 84, the mean GSI of females was significantly higher in the CON group (3.75 ± 1.31) than in 2CP:1AP and, on day 112, compared to other experimental treatments (7.99 ± 3.02) (Figure 4). At the end of the experiment, females with lowest GSI were found in 2CP:1AP and

1CP:2AP groups ($p < .05$) (Figure 4). The CON males showed significantly higher mean GSI compared experimental groups on day 84, and lower mean GSI than in the 1CP:2AP group on day 140 ($p < .05$) (Figure 5).

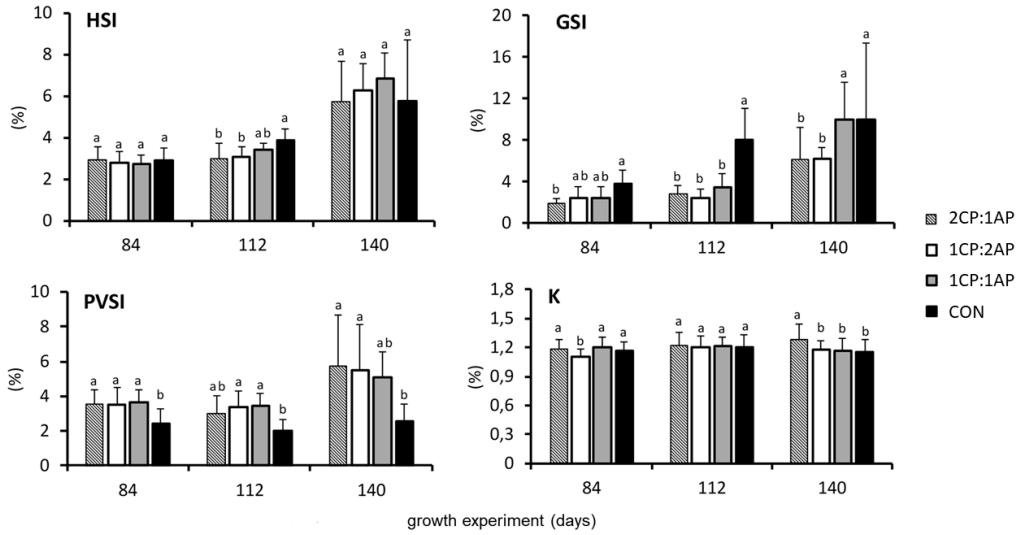

Figure 4. *Hepatosomatic index (HSI, %), gonadosomatic index (GSI, %), perivisceral fat index (PVSI, %), and condition factor (K) of female brook trout reared under three non-circadian photoperiods (see Figure 1 for details). Data are mean (bars) ± SD (whiskers). Different letters above bars indicate significant difference among treatments at a sampling date (p < .05).*

The mean HSI of females was significantly higher in the CON group compared with 2CP:1AP and 1CP:2AP on day 112 (3.89 ± 0.53) (Figure 4). The opposite was observed in males, in which HSI of controls was lower than in the treatment groups on day 112 ($p < .05$) (Figure 5). Both sexes showed significantly lower PVSI in the CON group throughout the experiment (Figures 4, 5) (Table 2).

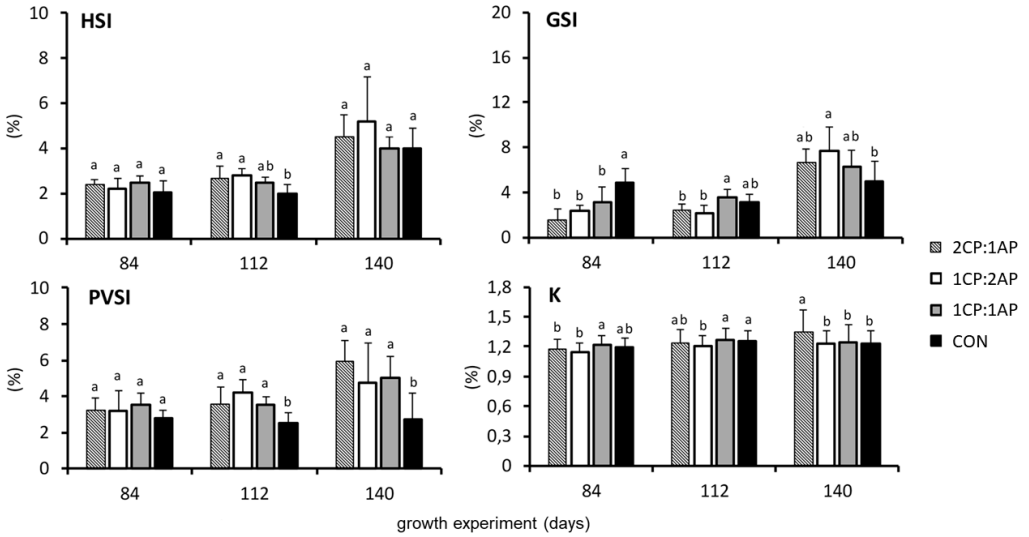

Figure 5. *Hepatosomatic index (HSI, %), gonadosomatic index (GSI, %), perivisceral fat index (PVSI, %), and condition factor (K) of male brook trout reared under three non-circadian photoperiods (see Figure 1 for details). Data are mean (bars) ± SD (whiskers). Different letters above bars indicate significant difference among treatments at a sampling date (p < .05).*

Table 2. *Summary of statistical model for hepatosomatic index (HSI), gonadosomatic index (GSI), perivisceral fat index (PVSI), condition factor (K) and fillet yield (FY) of brook trout reared under three non-circadian photoperiods.*

Parameter	Sampling day	Test type	Chi-squared / F-value	df	p-value
		Females			
HSI	84	ANOVA	0.339	3	0.797
	112	ANOVA	4.941	3	**0.007**
	140	ANOVA	0.607	3	0.615
GSI	84	Kruskal-Wallis	8.7	3	**0.034**
	112	Kruskal-Wallis	17.592	3	**0.0005**
	140	Kruskal-Wallis	8.404	3	**0.038**
PVSI	84	ANOVA	3.307	3	**0.032**
	112	ANOVA	4.996	3	**0.006**
	140	ANOVA	4.095	3	**0.014**
K	84	Kruskal-Wallis	41.761	3	**< 0.001**
	112	Kruskal-Wallis	2.408	3	0.492
	140	Kruskal-Wallis	40.922	3	**< 0.001**
Fillet yield	84	ANOVA	5.945	3	**0.006**
	112	ANOVA	15.77	3	**< 0.001**
	140	ANOVA	5.607	3	**0.004**

		Males			
HSI	84	ANOVA	1.946	3	0.143
	112	ANOVA	5.721	3	**0.003**
	140	Kruskal-Wallis	5.279	3	0.153
GSI	84	ANOVA	13.45	3	**< 0.001**
	112	ANOVA	8.789	3	**0.0002**
	140	ANOVA	3.854	3	**0.018**
PVSI	84	Kruskal-Wallis	5.164	3	0.160
	112	ANOVA	8.08	3	**0.0004**
	140	ANOVA	6.647	3	**0.001**
K	84	Kruskal-Wallis	25.779	3	**< 0.001**
	112	Kruskal-Wallis	10.921	3	**0.012**
	140	Kruskal-Wallis	11.899	3	**0.008**
Fillet yield	84	ANOVA	0.843	3	0.517
	112	ANOVA	0.361	3	0.781
	140	ANOVA	2.016	3	0.133

Note. Model types given as subheadings; see main text and Figure 4, 5, 6 for details.
df – degrees of freedom. Significant values ($p < .05$) in bold.

On day 84, the mean K of females was significantly lower in the 1CP:2AP group (1.11 ± 0.08), and, at the end of the study, the 2CP:1AP presented a higher K (1.28 ± 0.16) than in all other treatments and controls ($p < .05$) (Figure 4). The same results at the end of the growth experiment were found for the 2CP:1AP male group (Figure 5). Fillet yield was significantly lower in the female CON group compared to all experimental groups (Figure 6).

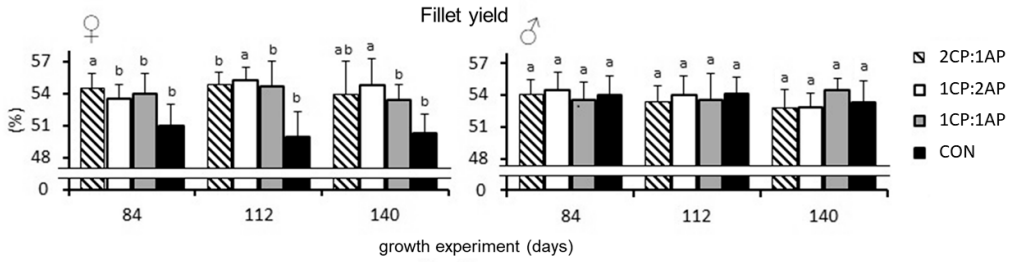

Figure 6. *Fillet yield (%) of brook trout reared under three non-circadian photoperiods (see Figure 1 for details). Data are mean (bars) ± SD (whiskers). Different letters indicate significant difference among treatments at a sampling date (p < .05).*

On days 84 and 112, testosterone was higher in CON females and males than in the experimental groups ($p < .05$) (Figure 7). On day 140, the highest testosterone levels were observed in 1CP:1AP males, and highest oestradiol in 1CP:1AP females (Figure 7). A similar pattern was observed for oestradiol among females, where at the end of the study oestradiol was significantly higher in 1CP:1AP (Figure 7). Similarly, changes in glucose were observed regardless of sex. On day 112, glucose was higher in 1CP:2AP than in CON and 2CP:1AP in both females and males. At the end of the experiment CON showed the lowest levels of glucose among treatments ($p < .05$) (Figure 7) (Table 3).

Table 3. *Summary of statistical model for glucose, testosterone, and oestradiol concentrations in blood plasma of female and male brook trout reared under three non-circadian photoperiods.*

Parameter	Sampling day	Test type	Chi-squared / F-value	df	p-value
		Females			
Testosterone	84	Kruskal-Wallis	14.375	3	**0.002**
	112	Kruskal-Wallis	19.098	3	**0.0003**
	140	Kruskal-Wallis	8.483	3	**0.037**
Oestradiol	84	Kruskal-Wallis	16.157	3	**0.001**
	112	Kruskal-Wallis	20.604	3	**0.0001**
	140	Kruskal-Wallis	14.316	3	**0.003**
Glucose	84	Kruskal-Wallis	8.889	3	**0.031**
	112	Kruskal-Wallis	12.326	3	**0.006**
	140	Kruskal-Wallis	21.437	3	**< 0.001**
		Males			
Testosterone	84	ANOVA	40.14	3	**< 0.001**
	112	Kruskal-Wallis	22.345	3	**< 0.001**
	140	Kruskal-Wallis	21.234	3	**< 0.001** 0
Oestradiol	84	ANOVA	3.414	3	**0.034**
	112	ANOVA	2.298	3	0.103
	140	ANOVA	7.263	3	**0.002**
Glucose	84	ANOVA	5.656	3	**0.004**
	112	ANOVA	1.844	3	0.166
	140	Kruskal-Wallis	17.621	3	**0.0005**

Note. Model types given as subheadings; see main text and Figure 7 for details. df – degrees of freedom. Significant values ($p < .05$) in bold.

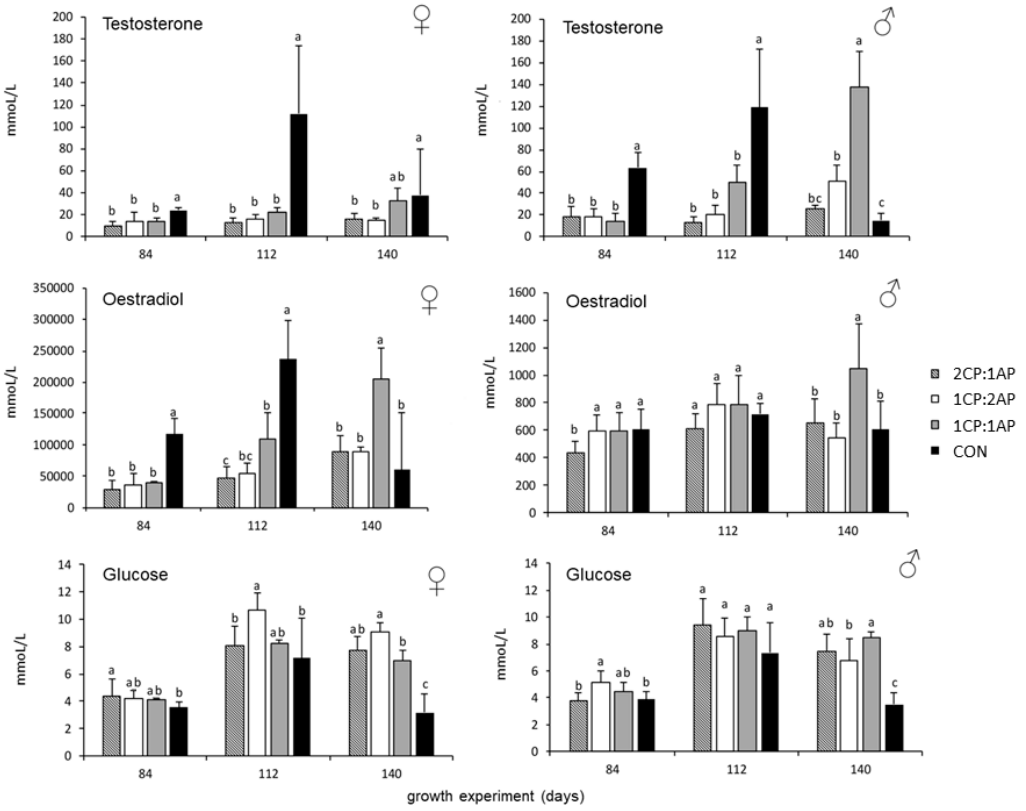

Figure 7. *Glucose, testosterone, and oestradiol concentrations in blood plasma of female and male brook trout reared under three non-circadian photoperiods (see Figure 1 for details). Data are mean (bars) ± SD (whiskers). Different letters indicate significant difference among treatments at a sampling date (p < .05).*

The highest proportion of mature fish, based on abdominal palpation, was 80% in CON males and 29% in CON females. A significantly lower rate of mature males was observed in 1CP:1AP and 2CP:1AP groups (54% and 13%, respectively). No fish from the 1CP:2AP group matured (Fig. 8). The lowest survival was observed in group 2CP:1AP at 92%.

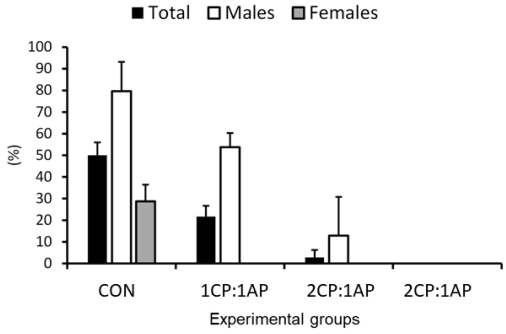

Figure 8. *Spawning readiness of brook trout reared under three non-circadian photoperiods (see Figure 1 for details). Data are mean (bars) ± SD (whiskers).*

The cost of maintaining the prolonged photoperiods was 25 € for 1CP:2AP, 37 € for 1CP:1AP, and 49 € for 2CP:1AP. Final calculations, including all expenses, revealed a cost kg^{-1} biomass of 7.7 ± 0.84 € for CON group, 7.8 ± 0.43 € for 1CP:1AP, 8.7 ± 0.35 € for 2CP:1AP, and 7.4 ± 1.12 € for 1CP:2AP.

Discussion

We found significant effects of a prolonged photoperiod on growth rate and time of puberty with both male and female controls showing significantly higher levels of preparedness for spawning, suggesting use of non-circadian photoperiod regime as an effective option in commercial fish culture.

Photoperiod is widely recognised as the major determining factor in sexual maturation of salmonids (Taranger et al., 1998). The seasonal timing of salmonid spawning can be manipulated with constant short or long photoperiods (Takashima and Yamada, 1984) and by condensing or extending the annual photoperiod cycle (Scott, 1990; Bromage et al., 1993). Moreover, factors such as temperature, photoperiod manipulation, and feeding strategy are able to delay sexual maturity to some degree (Iversen et al., 2016). However, there is limited information about non-circadian photoperiod on development and growth. Organisms reared under non-circadian cycles may suffer effects of disturbance to physiological processes (Dalley, 1980). Dalley (1980) suggested that exposure to environmental cycles with non-circadian periods might be beneficial to prawns, as life-history processes can be accelerated via compression of the organism's relative time-scale. Von Saint Paul and Aschoff (1978) and Saunders (1972) stated that non-circadian photoperiods exert a negative effect on growth and survival of some insects. It has been reported that rearing under non-circadian light regimes can cause increased mortality, reductions in growth, and developmental changes in the prawn Palaemon elegans (Dalley, 1979). Results of our study demonstrate a growth-promoting effect of a non-circadian photoperiod regimes. Our data of growth with respect to photoperiod manipulation of brook trout are in agreement with studies of sex steroid production in rainbow trout and Atlantic salmon *Salmo salar* (Taylor et al., 2005; Hansen et al., 2017). The growth rate of fish reared under artificially adjusted light regimes was higher than those reared under ambient light. In agreement with Randall et al. (2001) and Taylor et al. (2006), growth stimulation in the present study was not associated with the extended light period, but by increase of daylight hours. The effects of prolonged light regimes on growth during the typical spawning period can be explained by suppression of maturation and allocation of energy to somatic growth as opposed to gonad development which was confirmed by the observed among-group GSI differences during the spawning period, that were most pronounced in males. The male CON group showed increased GSI during the pre-spawning period, with a decrease after spawning. For a limited period of time, the GSI of the experimental groups began to increase. A similar trend was observed among females. The chief role of sex steroids is the regulation of gametogenesis via the synthesis of vitellogenin and the proliferation of spermatogonia during spermatogenesis (Qiu et al., 2015). We observed significantly higher testosterone and oestradiol in male and female CON, with a lower level of these sex steroids levels in experimental groups, indicating a delay of puberty. A sharp decrease in the level of testosterone and oestradiol was seen over the course of the experiment. Studies of salmonids report a reduction and return to baseline concentrations in oestradiol immediately before ovulation (Pavlidis et al., 1994; Frantzen et al., 2004). Our finding of significantly higher oestradiol in females than in males is consistent with previous studies (Mayer et al., 1990). Testosterone's effect on gonadotropin production and its role as precursor to oestrogens explains elevated plasma testosterone in females (Schulz and Miura,

2002). Analyses of testosterone and its changes in males are in agreement with reported results for brook trout and other salmonids (Holcombe et al., 2000; Qiu et al., 2015; Lundova et al., 2019). We conclude that fish maturation is delayed by a prolonged photoperiod under non-circadian regimens, as indicated by data of GSI, sex steroid levels, and spawning readiness of fish during the natural spawning period. These results can be compared with those of previous studies revealing that several photoperiodic species undergo spontaneous gonad regression during rearing under stimulatory day lengths (Kumar, 1997; Dawson et al., 2001) and show changes in responsiveness to long daylight periods that do not reach the length of the experimental stimulating day length (Budki et al., 2012). We successfully applied non-circadian photoperiods to improve the growth performance of brook trout, regardless of sex, by delaying sexual maturation. Lower production costs can be achieved by employing the technique for as short a period as necessary. Our period of 46 days of artificial lighting during 140 rearing days showed a positive effect. We recommend the use of 48 h natural ambient photoperiod alternating with 24 h of constant light to optimize growth rate, reduce GSI, and produce fewer sexually mature fish at the lowest cost, while avoiding deterioration in flesh quality and fish loss, and maintaining fish welfare.

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Compliance with ethical standards

Experimentation was conducted in compliance with Czech law and EU regulations for the care and use of laboratory animals Directive 2010/63/EU.

Conflict of interest

The authors declare that they have no conflict of interest

References

Bromage, N., Randall, C., Duston, J., Thrush, M., Jones, J., 1993. Environmental control of reproduction in salmonids. In: Muir, J.F., Roberts, R.J. (Ed.), Recent Advances in Aquaculture 4. Oxford: Blackwell Scientific Publications, pp. 55–65.

Brown, E.E., Baumann, H., Conover, D.O., 2014. Temperature and photoperiod effects on sex determination in a fish. J. Exp. Mar. Bio. Ecol. 461, 39–43.

Budki, P., Rani, S., Kumar, V., 2012. Persistence of circannual rhythms under constant periodic and aperiodic light conditions: sex differences and relationship with the external environment. J. Exp. Biol. 215, 3774–3785.

Dalley, R., 1979. Effects of non-circadian light cycles on the survival and development of *Palaemon elegans* Rathke reared in the laboratory. In: Naylor, E., Hartnoll, R.G. (Ed.), Cyclic phenomena in marine plants and animals. Proc. 13th Europ. Mar. Biol. Symp. Pergamon Press, London, pp. 157–163.

Dalley, R., 1980. The survival and development of the shrimp *Crangon crangon* (L.), reared in the laboratory under non-circadian light-dark cycles. J. Exp. Mar. Biol. Ecol. 47, 101–112.

Dawson, A., King, V.M., Bentley, G.E., Ball, G.F., 2001. Photoperiodic control of seasonality in birds. J. Biol. Rhythms 16, 365–380.

Fjelldal, P.G., Imsland, A., Hansen, T., 2012. Vaccination and elevated dietary phosphorus reduces the incidence of early sexual maturation in Atlantic salmon (*Salmo salar* L.). Aquaculture 364, 333–337.

Frantzen, M., Arnesen, A.M., Damsgård, B., Tveiten, H., Johnsen, H.K., 2004. Effects of photoperiod on sex steroids and gonad maturation in Arctic charr. Aquaculture 240, 561–574.

Hansen, T.J., Fjelldal, P.G., Folkedal, O., Vagseth, T., Oppedal, F., 2017. Effects of light source and intensity on sexual maturation, growth and swimming behaviour of Atlantic salmon in sea cages. Aquac. Environ. Interact. 9, 193–204.

Harris, J., Bird, D.J., 2000. Modulation of the fish immune system by hormones. Vet. Immunol. Immunopathol. 77, 163–176.

Holcombe, G.W., Pasha, M.S., Jensen, K.M., Tietge, J.F., Ankley, G.T., 2000. Effects of photoperiod manipulation on brook trout reproductive development, fecundity, and circulating sex steroid concentrations. N. Am. J. Aquac. 62, 1–11.

Iversen, M., Myhr, A.I., Wargelius, A., 2016. Approaches for delaying sexual maturation in salmon and their possible ecological and ethical implications. J. Appl. Aquacult. 28, 330–369.

Jonsson, B., Finstad, A.G., Jonsson, N., 2012. Winter temperature and food quality affect age at maturity: An experimental test with Atlantic salmon (*Salmo salar*). Can. J. Fish Aquat. Sci. 69, 1817–1826.

Kumar, V., 1997. Photoperiodism in higher vertebrates: an adaptive strategy in temporal environment. Indian J. Exp. Biol. 35, 427–437.

Linhartova, Z., Lunda, R., Masilko, J., Dvorak, P., Lundova, K., Stejskal, V., Matousek, J., Mraz, J., 2018. Impact of photostimulation for delayed maturity on flesh quality of brook trout (*Salvelinus fontinalis*) stored under refrigerated conditions. Aquacult. Res. 49, 3817–3829.

Lundova, K., Matousek, J., Prokesova, M., Vanina, T., Sebesta, R., Urban, J., Stejskal, V., 2019. The effects of a prolonged photoperiod and light sources on growth, sexual maturation, fin condition, and vulnerability to fungal disease in brook trout *Salvelinus fontinalis*. Aquacult. Res. 50, 256–267.

Lundova, K., Matousek, J., Prokesova, M., Sebesta, R., Policar, T., Stejskal, V., 2019. The effect of timing of extended photoperiod on growth and maturity of brook trout (*Salvelinus fontinalis*). Aquacult. Res. 50, 1697–1704.

Mayer, I., Lundqvist, H., Bergulund, I., Schmitz, M., Schulz, R., Borg, B., 1990. Seasonal endocrine changes in Baltic salmon, *Salmo salar*, immature parr and mature male parr. I. Plasma levels of five androgens, 17a-hydroxy-20-b-dihydroprogesterone, and 17-b-estradiol. Can. J. Zool. 68, 1360–1365.

Noori, A., Amiri, B.M., Mirvaghefi, A., Rafiee, G., Neitali, B.K., 2015. Enhanced growth and retarded gonadal development of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum) following a long-day photoperiod. Aquacult. Res. 46, 2398–2406.

Önder, M.Y., Basçınar, N., Khan, U., Sonay, F.D., 2016. Effect of photoperiod on growth and efficiency of yolk-sac utilization in alevins of Brook Trout (*Salvelinus fontinalis*). Pakistan J. Zool. 48, 533–537.

Pankhurst, N.W., Munday, P.L., 2011. Effects of climate change on fish reproduction and early life history stages. Mar. Freshwater Res. 62, 1015–1026.

Pavlidis, M., Dimitriou, D., Dessypris, A., 1994. Testosterone and 17-b-estradiol plasma fluctuations throughout spawning period in male and female rainbow trout, *Oncorhynchus mykiss* (Walbaum), kept under several photoperiod regimes. Ann. Zool. Fennici 31, 319–327.

Qiu, D., Xu, S., Song, C., Chi, L., Li, X., Sun, G., Liu, B., Liu, Y., 2015. Effects of spectral composition, photoperiod and light intensity on the gonadal development of Atlantic salmon *Salmo salar* in recirculating aquaculture systems (RAS). Chin. J. Ocean Limnol. 33, 45–56.

Randall, C.F., North, B., Futter, W., Porter, M., Bromage, N., 2001. Photoperiod effects on reproduction and growth in rainbow trout. Trout News 32, 12–16.

Saunders, D.S., 1972. Circadian control of larval growth rate in *Sarcophaga argyrostoma*. Proc. Nation. Acad. Sci. U.S.A. 69, 2738–2740.

Schulz, R.W., Miura, T., 2002. Spermatogenesis and its endocrine regulation. Fish Physiol. Biochem. 26, 43–56.

Schulz, R.W., Andersson, E., Taranger, G.L., 2006. Photoperiod manipulation can stimulate or inhibit pubertal testis maturation in Atlantic salmon (*Salmo salar*). Anim. Reprod. 3, 121–126.

Scott, A.P., 1990. Salmonids. In: Munro, A.G., Scott, A.P., Lam, T.J, (Ed.) Reproductive Seasonality in Teleosts: Environmental Influences. CRC Press, Boca Raton, pp 33–53.

Skjæraasen, J.E., Salvanes, A.G.V., Karlsen, Ø., Dahle, R., Nilsen, T., Norberg, B., 2004. The effect of photoperiod on 277 sexual maturation, appetite and growth in wild Atlantic cod (*Gadus morhua* L.). Fish Physiol. Biochem. 30, 163–174.

Svinger, V., Policar, T., Steinbach, C., Polakova, S., Jankovych, A., Kouril, J., 2013. Synchronization of ovulation in brook char (*Salvelinus fontinalis*, Mitchill 1814) using emulsified d-Arg6Pro9Net sGnRHa. Aquacult. Int. 21, 783–799.

Stien, L.H., Bracke, M.B.M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P.J., Vindas, M.A., Overli, O., Kristiansen, T.S., 2013. Salmon welfare index model (SWIM 1.0): A semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. Rev Aquacult 5, 33–57.

Takashima, F., Yamada, Y., 1984. Control of maturation in masu salmon by manipulation of photoperiod. Aquaculture 43, 243–257.

Taranger, G.L., Haux, C., Stefansson, S.O., Bjornsson, B.T., Walther, B.Th., Hansen, T., 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17β profiles in Atlantic salmon, *Salmo salar*. Aquaculture 162, 85–98.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E., Hausen, T., 2010. Control of puberty in farmed fish. Gen. Comp. Endocrinol. 165, 483–515.

Taylor, J.F., Migaud, H., Porter, M.J.R., Bromage, N.R., 2005. Photoperiod influences growth rate and insulin-like growth factor-I (IGF-I) levels in juvenile rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 142, 169–185.

Taylor, J.F., North, B.P., Porter, M.J.R., Bromage, N.R., Migaud, H., 2006. Photoperiod can be used to enhance growth and improve feeding efficiency in farmed rainbow trout, *Oncorhynchus mykiss*. Aquaculture 256, 216–234.

Thorpe, J., Mangel, M., Metcalfe, N., Huntingford, F., 1998. Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. Evol Ecol 12, 581–599.

Türker, A., Yıldırım, Ö., 2011. Interrelationship of photoperiod with growth performance and feeding of seawater farmed rainbow trout (*Oncorhynchus mykiss*). Turk. J. Fish Aquat. Sci. 11, 393–397.

Von Saint Paul, U., Aschoff, J., 1978. Longevity among blowflies *Phormia terraenovae* R.D. kept in non-24 hour light/dark cycles. J. Comp. Physiol. 127, 191–195.

Zajic, T., Mraz, J., Sampels, S., Pickova, J., 2016. Finishing feeding strategy as an instrument for modification of fatty acid composition of brook trout (*Salvelinus fontinalis*). Aquacult. Int. 24:1641–1656.

CHAPTER 5

IMPACT OF PHOTOSTIMULATION FOR DELAYED MATURITY ON FLESH QUALITY OF BROOK TROUT (*SALVELINUS FONTINALIS*) STORED UNDER REFRIGERATED CONDITIONS

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Impact of photostimulation for delayed maturity on flesh quality of brook trout (*Salvelinus fontinalis*) stored under refrigerated conditions

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Abstract

The artificial prolongation of daylight is generally used to delay sexual maturation in many cultured species, mainly in salmonids. This may have a negative impact on flesh quality. Therefore, we aimed to investigate the impact of photostimulation on post-mortem changes; sensory, textural and nutritional characteristics of flesh of brook trout, *Salvelinus fontinalis*. Farmed brook trout were randomly divided into four experimental groups, according to photoperiod and sex for 112 days. Fish body traits and flesh quality of experimental fish were examined by body weight (g), gonadosomatic, hepatosomatic and perivisceral fat indices (%), rigor index (%), pH changes, fillet contraction (%), gaping, flesh water losses, textural, colour and sensory analyses and finally fat content and fatty acid (FA) composition. The results of pH measurement and rigor mortis showed that lower values of pH were measured in fish under artificial photoperiod (AP) during the entire measurement with faster progress of rigor mortis in comparison to fish under natural photoperiod (NP, control). In colour measurement, significant differences were detected in lightness L^*, where NP fish (mature) were lighter than AP fish (immature). Significantly higher fillet contraction ($p < 0.05$) more than 7% were observed among AP females compared to the control groups. Finally, we documented that extension of light period has a positive effect on the sensory evaluation, the fat content and FA composition of brook trout flesh, but the effect is only minimal.

KEYWORDS
brook trout, fatty acid composition, flesh quality, photoperiod, post-mortem changes, sexual maturation

1 | INTRODUCTION

Sexual maturation is a physiological process during which energy is invested in gonadal development instead of body growth. The gonadal development occurs at the expense of stored energy with nutrients, including lipids being mobilised from somatic stores (Manor et al., 2012). This repartitioning alters growth efficiency and has a negative impact on muscle nutritional quality by the depletion of intramuscular fat and protein and changes in composition of important fatty acids (FA) in many cultured fish species (Manor, Weber, Cleveland, & Kenney, 2014; Salem, Silverstein, Rexroad, & Yao, 2007; Taranger et al., 2010). Also the flesh quality of farmed salmonids is known to change with season (Espe et al., 2004; Roth et al.,

2005), mostly due to the changes in sexual maturation, that alters the body morphology, muscle lipid content, texture, aftertaste and colour of the flesh (Aksnes, Gjerde, & Roald, 1986; Manor et al., 2014; Reid & Durance, 1992). This negative impact of sexual maturation on flesh quality and product marketability has been reported in several studies on cultured rainbow trout, *Oncorhynchus mykiss* (Walbaum), where sexual maturation resulted in reduction of flesh quality—softer fillets with reduced fat, decrease of bright red colour and low slaughter yield. This is not demanded by the market, where consumers are used to buy standard rainbow trout fillets—oily and of bright red colour (Cleveland, Kenney, Manor, & Weber, 2012; Paaver, Gross, & Ilves, 2004; Salem, Kenney, & Rexroad, 2006). Moreover, Gorgun and Akpinar (2007) reported significant differences in specific fatty acids between mature and immature female rainbow trout. Also, reduced flesh quality and distinctive skin colouration of sexually mature Atlantic salmon, *Salmo salar* L., were reported by Michie (2001) and resulted in loss of its demand on the market. Sexually mature salmonids are immunosuppressed that leads to the increased susceptibility to disease resulting in higher mortality rate during on-growing (Bruno, 1989; Currie & Woo, 2007; Traxler, Roome, Lauda, & LaPatra, 1997). In summary, the occurrence of sexual maturity in cultured salmonids represents a risk for their health, growth, feeding performance and flesh quality (Leclercq, Taylor, Hunter, & Migaud, 2010).

Generally, variation in flesh quality and texture of farmed fish is not only associated with sexual maturation (described above). This is influenced by a number of different external factors, such as feeding (Einen, Mørkøre, Rørå, & Thomassen, 1999), light regime (described below), starvation (Einen &Thomassen, 1998; Einen et al., 1999; Mørkøre, Mazo, Tahirovic, & Einen, 2008), body size (Veland & Torrissen, 1999), seasonality (Roth et al., 2005) etc. Or factors having consequences on flesh quality e.g., stress (Kristoffersen, Tobiassen, Steinsund, & Olsen, 2006)—mainly the pre-slaughter stress (Mørkøre et al., 2008), pre- or post-rigor filleting (Kristoffersen, Vang, Larsen, & Olsen, 2007) and muscle fibre density, where higher fibre density is related to a reduction in gaping and an increase in flesh firmness (Johnston et al., 2000, 2002, 2004). Fillet gaping has attracted the focus during the past three decades and has been reported for several fish species (Fletcher, Hallett, & Holland, 1997; Foss et al., 2009; Hagen & Johnsen, 2016); however, not for brook trout, *Salvelinus fontinalis* (Mitchill). It is defined as failure of the connective tissue and muscle segments to holes, which makes the visual appearance of the fillet less attractive (Hagen & Johnsen, 2016). Also, lipids affect the quality attributes of fish flesh, mainly by the length of chain and the degree of saturation of specific FA. The unsaturated FA are susceptible to autoxidation that affects firmness, off-flavour and colour development of fish flesh (Manor et al., 2012; Wood et al., 2003). In particular, polyunsaturated fatty acids (PUFA), especially $n-3$ highly unsaturated fatty acids ($n-3$ HUFA), the eicosapentaenoic (EPA; C20:5$n-3$) and docosahexaenoic (DHA; C22:6$n-3$) FA, found mainly in fatty fishes such as salmonids, have a beneficial effect on human health, especially, in the prevention of human coronary disease and weight reduction. Moreover, they provide a healthy and a balanced ratio of $n-6$ and $n-3$ FA (Adamkova et al., 2011; Kris-Etherton, Harris, & Appel, 2002; Lund, 2013). The increased intake of $n-3$ HUFA (EPA and DHA) has shown to reduce the incidence of cardiovascular, coronary artery diseases, cancers and diabetes (Adamkova et al., 2011; Brouwer, Geelen, & Katan, 2006; Simopoulos, 2002).

It is well known that photoperiod stimulates endogenous physiological rhythms that regulate appetite and growth (Smith, Metcalfe, Huntingford, & Kadri, 1993), and synchronise sexual maturation and reproduction in fish in temperate regions (Johnston et al., 2004; Taranger et al., 1998, 2010). The responses of fish to photostimulation are dependent on their nutritional and physiological state and the timing of photoperiod changes (Hansen et al., 2001). In many cultured species, including salmonids, artificial prolongation of the day is generally used to delay the sexual maturation. For example, the on-growing salmon industry routinely applies artificial continuous light during the second-year at sea (Endal, Taranger, Stefansson, & Hansen, Taranger, Stefansson & Hansen, 2000; Hansen, Stefansson, & Taranger, 1992) or during smoltification with constant light regime until early summer by achieving significant improvements in growth performance (Duncan, Mitchell, & Bromage, 1999; Johnston et al., 2004). This is strengthened by several studies that have reported how the photoperiod manipulation positively alter the normal spawning period of different salmonid species such as Atlantic salmon (Johnston et al., 2004; Johnston, Hambrook, Gray, & Davidson, 1992), rainbow trout (Bromage, Elliot, Springate, & Whitehead, 1984; Whitehead & Bromage, 1980), masu salmon, *Oncorhynchus masou* (Takashima & Yamada, 1984) and also brook trout (Henderson, 1963; Holcombe, Pasha, Jensen, Tietge, & Ankle, 2000; McCormick & Naiman, 1984). Therefore, photoperiod regime is successfully applied in practice and seems to be effective for growth enhancement and delaying sexual maturation (Endal et al., 2000; Hansen et al., 1992; Taranger et al., 1998, 1999). However, the effect of different photoperiod regiments on texture and nutritional quality of flesh in brook trout has not been investigated yet.

The main objective of this study was to evaluate and compare the effect of photoperiod alteration on post-mortem changes, sensory, textural and nutritional characteristics of brook trout flesh.

2 | MATERIALS AND METHODS

2.1 | Experimental fish and filleting

The present work reflects the study of Lundova et al. (personal communication) about the effect of photostimulation timing on growth and puberty in brook trout. Four experimental groups (according to photoperiod and sex) of brook trout of marketable size (219–336 g, 2-year-old) were intensively cultured in six outdoor plastic rectangular flow through tanks (three tanks/photoperiod group without sex selection) and fed with commercial feed BioMar EFICO Alpha 756 (BioMar A/S, Brand, Denmark) at fish farm Litomyšl s.r.o., Czech

Republic. Each tank (2.7 m^3, 300 × 100 × 90 cm; water flow rate 3–3.5 m^3 h^{-1}) was stocked with 150 fish resulting in initial biomass 27.6 ± 2.1 kg m^3. Initial body weight and total length for AP group were 188.6 ± 38.6 g and 254 ± 17 mm respectively. Initial body weight and total length for NP group were 195.2 ± 45.6 g and 255 ± 18 mm respectively. Final survival rate reached 71.8 ± 15.6 and 68.2% ± 3.2% in AP and NP group respectively. Fish were fed by hand twice per day (at 0,800 and 1,500 hr) according to actual appetite (till to stop the feeding behaviour). The nutritional composition of the feeding is listed in Table 1. Feeding was stopped 48 hr before harvesting. Water parameters (pH, temperature, dissolved oxygen) and fish mortality were monitored twice per day. The experimental groups differed in sex and photoperiod regime: APF—females exposed to artificially prolonged photoperiod starting from 6 August (46 days after the summer solstice); APM—males exposed to artificially prolonged photoperiod starting from 6 August (46 days after the summer solstice); NPF—females reared under natural ambient photoperiod; NPM—males reared under natural ambient photoperiod. In artificial photoperiod (AP) treatments, the natural day length was prolonged to 18 hr of daylight (18L:6D) using the light emitting diodes (LED) for 112 days, whereas in natural photoperiod treatments (NP, control), fish were contained under natural day length. Each tank in AP treatment was equipped with two LED lamps—Epistar 50 W LED CO8 (50 W, 4,000 lm, 6,000 K) (Epistar, China) with light intensity from 250 to 1,000 lux (depending on the distance from the light source). Light intensity in water was monitored with submersible photometer for aquaculture Hydrolux (BGB Innovation Ltd., Lincolnshire, United Kingdom). Clove oil (0.03 ml L^{-1}) was used as an anaesthetic for limiting the stressful manipulation for fish during all control measurements. Body weight, blood sampling, weight of gonads, livers and perivisceral fat were measured three times during the 112-day experiment (56th, 84th and 112st day) to document the changes in time (Figure 1a–d) which is described in detail in the study of Lundova et al. (personal communication). After 112 days, 80 fish were randomly selected and divided into four experimental groups—namely APF, APM, NPF and NPM (n = 20 per treatment). The maturity of each fish was tested by stripping or massaging the abdominal part. Then fish were weighed, after using an over-dose of anaesthetic, slaughtered by blows to the head, bled and gutted. After gutting, gonads, livers and perivisceral fat were separated and weighed. Individual gutted fish were washed in cold water with ice, packed separately in plastic bags, and stored on ice. Fish selected for fillets were hand filleted by the same person to ensure consistency. Fish were immediately transported on ice to the processing facilities of the Institute of Aquaculture and Protection of Waters (IAPW), Faculty of Fisheries and Protection of Waters (FFPW), University of South Bohemia (USB) in České Budějovice, Czech Republic. Packed fillets were stored in straight horizontal position separately in a refrigerated chamber at a temperature of 2.0 ± 0.5°C for 5 days for further analyses. Fillet yield, gonadosomatic (GSI), hepatosomatic (HSI) and perivisceral fat (PFI) indices in percentage were calculated according to formulas:

$$\text{Fillet yield} = ((\text{Right} + \text{Left fillet weight})/\text{Fish weight}) \times 100,$$

$$\text{GSI} = (\text{Gonad weight}/\text{Fish weight}) \times 100,$$

$$\text{HIS} = (\text{Liver weight}/\text{Fish weight}) \times 100,$$

$$\text{PFI} = (\text{Perivisceral fat weight}/\text{Fish weight}) \times 100$$

TABLE 1 Nutritional composition (%, MJ/kg) of feeding (pellets of diameter 3–4.5 mm) BioMar EFICO Alpha used during experiment

Nutrition composition	Unit	Value
N-compounds	%	39–42
Lipids	%	21–24
Carbohydrates (NFE)	%	21
Fibre	%	4.7
Ash	%	5.9
Total phosphorus	%	0.8
Gross energy	MJ/kg	20–24
Digestible energy	MJ/kg	18.7

2.2 | Rigor index

Rigor index (RI) was evaluated on gutted fish (n = 20 per experimental group; F + M together because of no significantly different results between sex) stored in a refrigerated chamber at 2.0 ± 0.5°C over the period of 61 hr. The development of RI was regularly monitored every 3 hours by placing the fish on a horizontal table and measuring the distance of the base of the caudal fin from the horizontal line of the table by a vertical scale ruler. Fish were stored flat between measurements. Full rigor was attained when the fish was extremely stiff and rod like (RI = 100%). RI (%) was calculated according to Bito (1986):

$$\text{RI} = 100 \times (\text{distance of caudal fin at time } 0 - \text{distance of caudal finduringexperiment})/\text{distance of caudal fin at time } 0$$

2.3 | Muscle pH

The pH was measured by penetration of a probe pH2 of a digital pH meter Testo 206 (Testo s.r.o. Prague, Czech Republic) 2 cm into the dorsal muscle of right fillets (n = 10 per treatment, F + M together —no significant differences between sex) always at the same position. Probe was cleaned with distilled water and wiped between each measurement. Muscle pH was documented regularly every third hour over the period of 50 hr.

2.4 | Fillet contraction

Relative contraction (% of initial area) of brook trout fillets was analysed. For that, fresh (immediately after filleting) and post-rigor (60 hr

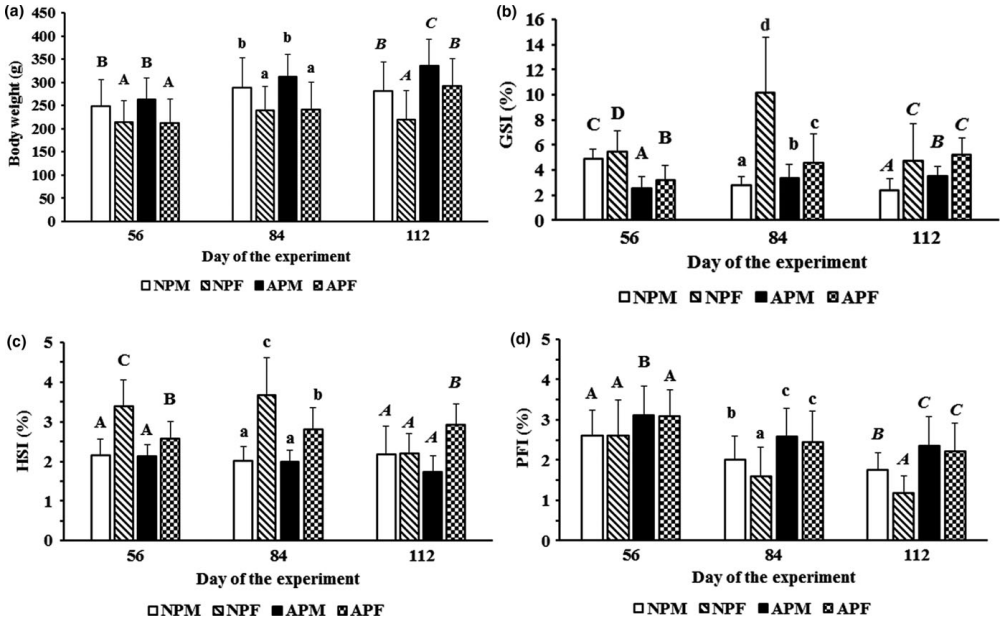

FIGURE 1 (A) Body weight (g), (B) GSI (%), (C) HSI (%) and (D) PFI (%) of brook trout of four experimental groups: artificial photoperiod female (APF); artificial photoperiod male (APM); natural photoperiod female (NPF); natural photoperiod male (NPM). Data are shown as mean (bars) ± standard deviation (whiskers) at 56th, 84th and 112th day of experiment. Bars with the same letter are not significantly different (Tukey´s HSD test, $p > 0.05$), $n = 20$ per treatment.

after filleting) fillets ($n = 5$ per treatment) were photographed to assess fillet shrinkage. The relative contraction of fillets was evaluated using the MicroImage software version 4.0 (Olympus Optical co. GMBH, Hamburg, Germany).

2.5 | Gaping

Gaping was evaluated on post-rigor fillets ($n = 10$ per treatment) on a scale from 0 to 5 according to Andersen, Stromsnes, Steinsholt, and Thomassen (1994) (0 = no gaping; 5 = extreme gaping with the fillet falling apart) on the fourth day of analyses.

2.6 | Drip, cooking and thawing losses

To determine drip (DL), cooking (CL) and thawing (TL) losses, three pieces of 10-g flesh from the dorsal part of post-rigor fillets ($n = 10$ per treatment) were dissected, weighed and packed separately under vacuum in polyvinyl chloride bags on the fourth day of analyses. Samples for DP were stored at 2.0 ± 0.5°C in a refrigerator for 24 hr. Packed samples for CL were cooked in a water bath at 75°C for 20 min and cooled down in a refrigerator for 3 hr. Packed samples for TL determination were frozen at −20°C for 24 hr and thawed to 4°C in a refrigerator. After 3/24 hr, samples were unpacked, exudate was wiped and samples were weighed. DL, CL and TL in percentage were calculated according to formulas:

$$\mathrm{DL} = (\text{weight of flesh at time } 0 - \text{weight of flesh after24hr})/(\text{weight of flesh at time } 0) \times 100,$$

$$\mathrm{CL} = (\text{weight of uncooked flesh} - \text{weight of cooked flesh})/(\text{weight of uncooked flesh}) \times 100,$$

$$\mathrm{TL} = (\text{weight prior to freezing} - \text{weight after thawing})/(\text{weight prior to freezing}) \times 100.$$

2.7 | Textural measurement

For textural profile analysis (TPA), post-(2)(1)rigor fillets ($n = 5$ per treatment) were used to measure hardness (puncture test) with a TA.XTPlus—pro Texture Analyzer from Stable Micro Systems (Godalming, Surrey, England). Samples (25 mm in diameter) were dissected from the mid-dorsal part of the fillet below the dorsal fin. A Dia Cylinder Delrin with a diameter of 10 mm was used as a probe and the setting was established as follows: pre-test and post-test speed of 10 mm s^{-1}, test speed of 1 mm s^{-1} until the probe compressed the fillet to 40% of its height.

2.8 | Colour measurement

Colour of post-rigor (60 hr after filleting) flesh (n = 10 per treatment) was measured at three locations along the dorsal part (frontal, mid and caudal) and two locations along the ventral part (frontal and caudal) of each fillet to represent the whole surface using a Colour Spectrophotometer CM-600d (Konica Minolta Inc., Japan). Colorimetric data represented as CIE L^* - whiteness, a^* - the red-green axis and b^* - the yellow-blue axis were measured directly on the fillets and each spot was measured in duplicates.

2.9 | Sensory evaluation

The sensory quality of post-rigor raw and cooked fillets was evaluated by a panel of 10 trained members from the staff of IAPW, FFPW, USB, České Budějovice, Czech Republic in individual cubicles to separate panellists from each other (ISO 8589, 2007) under controlled conditions of light, temperature and humidity. The evaluators were asked to avoid the following prior to the test: drinking coffee, eating spicy food, being too hungry or too full, smoking and wearing strong perfumes. Sensory analysis of whole raw fillets of brook trout was examined by the consistency (5 - firm; 1 - soft); colour/discolouration (5 - no discolouration; 1 - extreme discolouration), odour (5 - extremely desirable; 1 - extremely undesirable), and overall acceptability (5 - extremely acceptable; 1 - extremely unacceptable) according to Másílko, Zajíc, and Hlaváč (2015). Samples for cooking were composed of three small pieces of flesh (2 × 2 cm), each from a different part of fillet of the appropriate group, placed separately in 0.2 L glass jars with a lid labelled with random 3 or 4 digit codes and cooked for 15 min at 150°C in an electric oven. To comply the rules ISO 6658 (2005) and ISO 8589 (2007), no salt, oil, or spices were added. Samples of each group were served warm, in unopened jars and evaluation of each sample was performed twice under different coding with 30 min break between assessments. The panellists classified five parameters, namely odour, flavour, aftertaste, consistency and assessing of liking and were supposed to indicate the rating by assigning a point on a 100 mm unstructured hedonic scale (0 mm = very good fresh quality; 100 mm = spoiled quality). Points in hedonic scale were measured by a ruler, converted to numbers and statistically analysed.

2.10 | Fat content and fatty acid analyses

Whole left fillets with skin (n = 10 per treatment) were homogenised in a table blender to represent all edible parts in each sample. Lipid extraction was performed following the method of Hara and Radin (1978) with some modifications described by Mraz and Pickova (2009). Lipids were extracted from a 1-g homogenised sample. This sample was mixed with hexane-isopropanol (HIP, 3:2) and 6.67% Na_2SO_4 and centrifuged at 5,000× rpm for 5 min at 18°C. Lipid phase (upper one) was transferred to glass tube and evaporated under nitrogen atmosphere. Total lipid content was determined gravimetrically and recalculated into percentage. Fatty acid methyl esters (FAME) were analysed from a 2-mg sample of extracted lipids. FAME were prepared according to Appelqvist (1968) by methylation with NaOH and boron-trifluoride methanol-complex (BF3) and analysed by gas chromatography (GC) Trace Ultra (Thermo Scientific, Milano, Italy) on a BPX-70 50-m fused silica capillary column (diameter × thickness: 0.22 mm × 0.25 μm, 0.22 mm, AGE, Austin, TX) described by Eriksson and Pickova (2007). Fatty acids were identified by comparison with a GLC-68D standard mixture (Nu-Check Prep, Elysian, MN) and absolute amount of individual FA (%) was calculated by using an internal standard (21:0) (Nu-check Prep, Elysian, MN). Moreover, nutritional indexes: $n-3/n-6$ and $n-6/n-3$ polyunsaturated FA (PUFA) and polyunsaturated/saturated (P/S) FA were determined according to Department of Health and Social Security, Diet and cardiovascular disease (1984).

2.11 | Statistical analyses

All data were calculated as means ± standard deviations (*SD*) and analysis of variance (ANOVA) with subsequent post hoc comparisons using Tukey's honest significant difference (HSD) test was performed. Sensory analyses were evaluated using hierarchical ANOVA (assessors nested in treatments, assessments nested in assessors) using Fischer´s low significant difference (LSD) test. Probability values of $p < 0.05$ were considered as significant. All statistical analyses were performed using STATISTICA software (Version 12, StatSoft, Inc., 2013) for MS Windows.

3 | RESULTS AND DISCUSSION

3.1 | Fish sample and filleting

The bodyweight of fish (A), GSI (B), HSI (C), and PFI (D) of all analysed groups namely APF - artificial photoperiod female; APM - artificial photoperiod male; NPF - natural photoperiod female and NPM - natural photoperiod male during 112 days of analyses are presented in Figure 1 The fish cultured under NP grew to smaller sizes after 112 days of analyses and 87% (88% M and 87% F) were sexually mature in comparison to the group under AP, where only 7% (15% M and 0% F) were sexually mature. This is well documented by Figure 1a, where significant differences ($p < 0.05$) after 112 days of photoperiod regime were found between NP and AP of both sex and fish under AP reached higher weights, up to 20%. Figure 1b shows that the highest significant difference ($p < 0.05$) in GSI was achieved after 84 days of treatment in females. The PFI proved that fish under NP (both F and M) inserted the energy into gonadal growth, because the PFI values were significantly lower ($p < 0.05$) than in AP fish after 112 days of artificial lightning (Figure 1d). The filleting yields (%) of APF, APM, NPF and NPM were 53.6 ± 2.6, 54.4 ± 2.3, 52.7 ± 3.3 and 52.6 ± 3.2 respectively, resulting in a higher filleting yield for fish under AP, but without significant differences in comparison to control group (NP). The filleting yield of

gutted AP fish was significantly higher (>4%, $p < 0.05$) in comparison to the control group. This was caused by lower weight of internal organs, mostly gonads, as explained above. These results show that AP can delay the sexual maturation of brook trout and fish under AP inserted their energy into body growth instead of gonadal growth. Moreover, the mortality was higher in control (NP) fish (32%) in comparison to the fish under AP (20%), indicating that AP had positively reflected in the elimination of aggressive behaviour of fish and secondary mycotic infections.

3.2 | Rigor and pH changes

In the present study, the first signs of rigor onset were observed between 4 and 7 hr post mortem. Subsequently, the rigor strength gradually increased and maximum rigor in NP and AP fish (94% and 91%) was achieved after 22 hr. Thereafter, the rigor strength diminished and fully resolved after 60 hr post mortem in both analysed groups (Figure 2). Significant differences ($p < 0.05$) between NP and AP fish were found from 10 to 16, 25, 28 and 34 to 56 hr post mortem. Interestingly, the faster progress of rigor mortis was demonstrated for AP fish in comparison to NP, which was assumed to be vice versa, because NP fish were under higher levels of stress thanks to their aggressive behaviour associated with sexual maturation. This could be explained by the length of starvation, where NP fish had empty digestive tracts, due to non-feed intake during sexual maturation, whereas AP fish starved only for 48 hr before slaughtering. Einen and Thomassen (1998), Einen et al. (1999) and Mørkøre et al. (2008) documented that starvation before slaughtering reduces the metabolic rate of the muscle, which suppresses the lactate formation from glycogen during anaerobic glycolysis in post-mortem muscle, that improve the firmness of the flesh and reduced the rigor development. However, there was no difference in the total feed intake as it reached a level of 131 ± 2 and 123 ± 5 g tank^{-1}.in AP and NP group respectively. The faster rigor onset and development is linked to depletion of ATP as well as glycogen (Mørkøre, Hansen, & Rørvik, 2006). Our results are in line with the study of Mørkøre et al. (2008), who reported a linear overall relationship between ATP level and the rigor angle, muscle pH and fillet contraction of gutted Atlantic salmon.

The unexpected results of RI were in line with the development of muscle pH, where the lower values of pH were measured in fish under AP during the entire measurement with faster progress of rigor mortis (described above) in comparison to fish under NP (Figure 3). Similar results were reported in the study of Mørkøre et al. (2008) on Atlantic salmon, where the muscle pH of the starved/control group showed significantly the slowest reduction rate and the lowest value. In the present study, the muscle pH dropped down from initial values 7.2 and 6.9 to 6.5 and 6.4 for NP and AP fish respectively after 7 hr post mortem. Then, the pH value stayed relatively stable and started to slowly rise after 28 hr in both the experimental groups. Interestingly, the ultimate pH (=the lowest measured value) was reached earlier than the full rigor was achieved (around 22 hr post mortem) as well as in the studies of Berg, Erikson, and Nordtvedt (1997) and Mraz, Linhartova & Masilko (personal communication).

3.3 | Fillet contraction

As it is generally known, pre-rigor fish fillets can freely contract during post-mortem changes which result in shrinkage of fillet area, because the flesh is not attached to the skeleton. Therefore, the loss of initial fillet area (%) was measured 60 hr post mortem to assess fillet shrinkage and to find possible differences within experimental groups. Significantly higher fillet contraction ($p < 0.05$) more than 7% had AP females (27.89%) compared to the control groups (20.40% for NPF and 20.85% for NPM). No significant difference was found for AP males (22.17%) with less than 2% difference in comparison with control groups (Figure 4). It might be hypothesised that higher fillet contraction might be related with faster onset and progress of rigor mortis demonstrated for AP fish in comparison to NP as reported by Mørkøre et al. (2008). The relative contraction was in range as reported for other fish species such as Atlantic cod, *Gadus Morhua* L., (around 30%) (Mørkøre et al., 2006) and Atlantic salmon (15%–17%) (Mørkøre et al., 2008) with higher contraction rate for fed salmon compared with the starved group, but higher than common carp (*Cyprinus carpio* L.) with 8% fillet contraction (Mraz et al., personal communication).

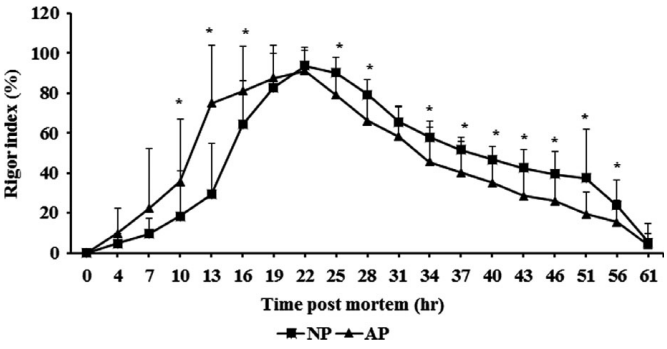

FIGURE 2 Development of rigor mortis evaluated by rigor index (%) in whole gutted brook trout stored at 2.0 ± 0.5°C during 61 hr. Data are mean ± standard deviation (*SD*) and * indicate statistical differences between AP and NP in time (Tukey´s HSD test, $p < 0.05$), $n = 20$ per experimental group (F + M together - no significant differences between sexes). AP: artificial photoperiod; NP: natural photoperiod.

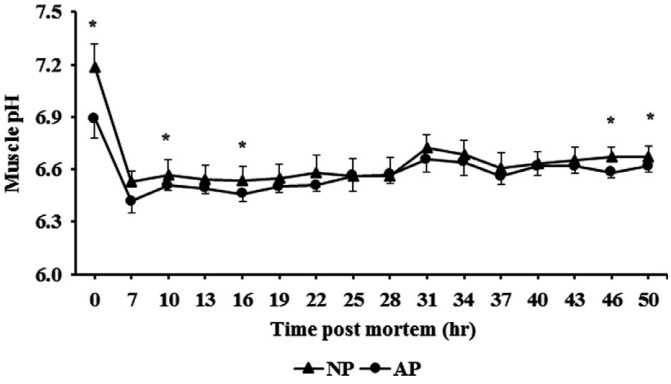

FIGURE 3 Development of muscle pH of brook trout fillets processed in pre-rigor and stored at 2.0 ± 0.5°C during 50 hr. Data are expressed as mean ± standard deviation (*SD*) and * indicate statistical differences between AP and NP in time, *n* = 10 per experimental group (F + M together - no significant differences between sex). AP: artificial photoperiod; NP: natural photoperiod

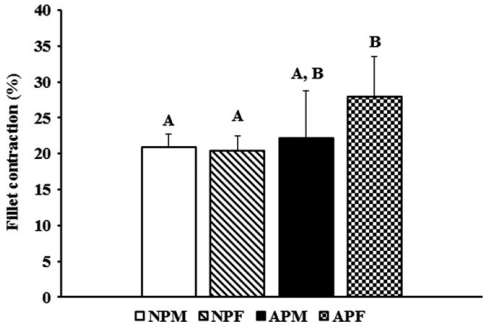

FIGURE 4 Relative contraction (% of initial area) of brook trout fillets (filleted in pre-rigor) after 60 hr storage at 2.0 ± 0.5°C. Bars are mean ± standard deviation (*SD*) and different letter above indicate statistical differences (Tukey´s HSD test, $p < 0.05$), *n* = 5 per treatment. AP: artificial photoperiod female; APM: artificial photoperiod male; NPF: natural photoperiod female; NPM: natural photoperiod male.

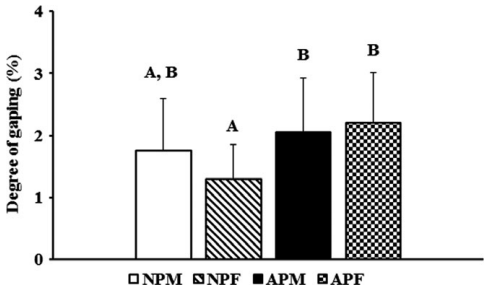

FIGURE 5 Degree of gaping (%) of brook trout post-rigor fillets stored at 2.0 ± 0.5°C. 0 = no gaping; 5 = extreme gaping. Bars are mean ± standard deviation (*SD*) and different letter above indicate statistical differences (Tukey´s HSD test, $p < 0.05$), *n* = 10 per treatment. APF: artificial photoperiod female; APM: artificial photoperiod male; NPF: natural photoperiod female; NPM: natural photoperiod male.

3.4 | Gaping and water losses

Gaping was observed in post-rigor fillets of all experimental groups (Figure 5). APF and APM groups exhibited a medium degree of gaping (2.2 ± 0.8 and 2.05 ± 0.9) and had softer texture compared to NPF with a significantly lower degree of gaping (only mild—1.3 ± 0.6). This may be due to the same reasons as described in fillet contraction. Moreover, similar to changes in texture (described below), gaping can be related to growth rate, state of maturity of the fish and rearing season (Espe et al., 2004; Lavety, Afolabi, & Love, 1988; Roth et al., 2005; Roth, Jenssen, Jonassen, Foss, & Imsland, 2007).

Water losses of brook trout post-rigor flesh (average fat content 7%) of all experimental groups are shown in Figure 6. Average drip losses were in the range of 2.04%–2.93% without significant differences ($p > 0.05$) among experimental groups. This was comparable with the studies of Varga et al. (2014) and Mraz et al. (personal

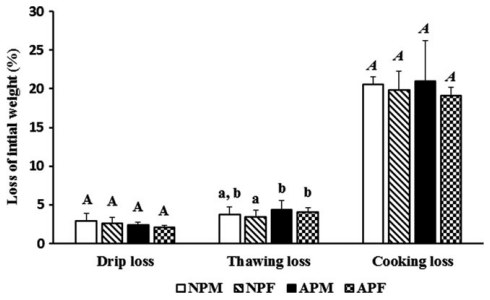

FIGURE 6 Drip, cooking and thawing losses (% loss of initial weight) of brook trout flesh dissected from dorsal part of post-rigor fillets. Results are shown as mean (bars) ± standard deviation (whiskers). Bars with the same letter are not significantly different (Tukey´s HSD test, $p > 0.05$), *n* = 10 per treatment. APF: artificial photoperiod female; APM: artificial photoperiod male; NPF: natural photoperiod female; NPM: natural photoperiod male

communication) on common carp, who reported drip losses (immediately after filleting) around 2.54%–2.79% and 3.7%, respectively. Thawing losses were in the range of 3.48%–4.32% and APM and APF had significantly higher values ($p < 0.05$) in comparison with NPF. Mraz et al. (personal communication) reported slightly lower thawing losses (1.8%) in carp post-rigor flesh, probably affected by variability in flesh texture among analysed fish, higher fat content (11.7%) and time of sampling (100 hr post mortem). Usually, the higher the fat content, the lower the water content is in the flesh. The highest water losses were achieved by cooking and were in the range of 19.13%–20.98% without significant differences ($p > 0.05$) among analysed groups. Comparable values were reported by Varga et al. (2014), who reported cooking losses of common carp around 22.19%–23.86%, than in the study of Mraz et al. (personal communication), who presented considerably lower values (14.7%). The possible explanation could be that Mraz et al. (personal communication). sampled the samples for CL determination 100 hr post mortem—much later than reported by Varga et al. (2014) (48 hr post mortem) and in the present study (72 hr post mortem), where the highest water losses are achieved in the time of full rigor and then gradually decrease.

TABLE 2 Texture parameters of fillets, hardness in 40% of fillet original thickness and fillet height (mm), of brook trout males/females treated under natural/artificial photoperiod

Brook trout males (M)/females (F) under natural (NP)/artificial (AP) photoperiod	texture parameters (mean ± *SD*)	
	Hardness (in 40%)	Height of fillets (mm)
NPM	405.65 ± 70.64^{b}	9.78 ± 0.86^{a,b}
NPF	301.71 ± 148.62^{a,b}	8.56 ± 0.90^{a}
APM	381.46 ± 101.88^{b}	10.67 ± 1.34^{b}
APF	223.32 ± 51.14^{a}	9.55 ± 0.91^{a,b}

Note. Data are represented as mean ± standard deviation (*SD*). Values followed by the same letters in the same column are not significantly different (Tukey´s HSD test, P > 0.05), $n = 5$ per treatment.
APF: artificial photoperiod female; APM: artificial photoperiod male; NPF: natural photoperiod female; NPM: natural photoperiod male.

3.5 | Textural and colour analyses

In the present study, higher values of hardness were observed in control fish in comparison with AP fish, in males by comparing with females, and significant differences were measured between APF (223.32 ± 51.14) with APM (381.46 ± 101.88) and NPM (405.65 ± 70.64) (Table 2). This could be associated with maturation of the fish, where control fish invested their energy in gonadal growth that resulted in lower fat content which may affect the textural quality as a softer consistency. This was reported by Andersen, Thomassen, and Rørå (1997) on rainbow trout, where lower fat content had higher resistance against compression, resulting in a firmer consistency of flesh. These differences in softness of fish flesh that occur during the period of rapid growth have been reported by many authors such as Lavety et al. (1988); Espe et al. (2004) and Roth et al. (2005), Roth et al. (2007). Not only growth rate connected with sexual maturation and fat content affect the texture proportion, also additional factors have an influence on textural

TABLE 3 Colour parameters of fillets represented as L^* - whiteness, a^* - redness and b^* - yellowness, of brook trout males/females treated under natural/artificial photoperiod

Colour parameters (mean ± *SD*)	Position	Brook trout males (M)/females (F) under natural (NP)/artificial (AP) photoperiod			
		NPM	NPF	APM	APF
L^*	1	55.56 ± 2.49^{b}	54.78 ± 3.14^{b}	49.66 ± 2.22^{a}	50.85 ± 2.11^{a}
	2	53.99 ± 3.22^{b}	55.12 ± 3.15^{b}	49.95 ± 2.07^{a}	49.03 ± 2.26^{a}
	3	54.80 ± 1.99^{b}	54.96 ± 2.37^{b}	50.07 ± 1.70^{a}	50.03 ± 1.96^{a}
	4	54.49 ± 2.98^{b}	57.15 ± 2.97^{c}	51.87 ± 3.24^{a}	53.13 ± 2.10^{a,b}
	5	54.96 ± 2.05^{b}	57.92 ± 3.94^{c}	51.74 ± 1.79^{a}	53.54 ± 2.37^{a,b}
a^*	1	−2.98 ± 0.49^{b}	−3.61 ± 0.39^{a}	−3.03 ± 0.44^{b}	−3.60 ± 0.60^{a}
	2	−3.29 ± 0.37^{a}	−3.27 ± 0.46^{a}	−2.90 ± 0.41^{a,b}	−3.19 ± 0.44^{b}
	3	−3.03 ± 0.54^{a}	−3.03 ± 0.47^{a}	−2.69 ± 0.48^{a}	−2.91 ± 0.55^{a}
	4	−1.71 ± 0.67^{a,b}	−1.97 ± 0.69^{a}	−1.23 ± 0.78^{b}	−1.61 ± 0.56^{a,b}
	5	−1.12 ± 0.74^{a,b}	−1.42 ± 0.63^{a}	−0.57 ± 0.58^{b}	−1.15 ± 0.86^{a,b}
b^*	1	6.91 ± 1.24^{b}	5.71 ± 1.33^{a}	4.78 ± 1.30^{a}	5.01 ± 1.51^{a}
	2	6.26 ± 0.89^{b}	6.62 ± 1.32^{b}	5.02 ± 1.48^{a}	4.83 ± 0.88^{a}
	3	7.01 ± 1.30^{b}	7.59 ± 0.98^{b}	5.84 ± 1.19^{a}	5.90 ± 1.19^{a}
	4	8.27 ± 0.90^{b,c}	9.03 ± 1.04^{c}	8.00 ± 1.17^{a,b}	7.29 ± 1.21^{a}
	5	8.34 ± 0.92^{a,b}	9.03 ± 0.95^{b}	7.69 ± 1.16^{a}	7.82 ± 1.39^{a}

Note. Data are shown as mean ± standard deviation (*SD*). Values with different letters in the same row are significantly different (Tukey´s HSD test, $p < 0.05$), $n = 10$ per treatment.
APF: artificial photoperiod female; APM: artificial photoperiod male; NPF: natural photoperiod female; NPM: natural photoperiod male

properties such as size of the fish, season (temperature), rearing conditions (Másílko et al., 2015; Stejskal et al., 2011) and thickness of fish fillet (Veland & Torrissen, 1999), which have to be taken into account.

Significant differences in colour were detected in lightness L^*, where NP fish (mature) were lighter than AP fish (immature) at all three locations measured along the dorsal part (positions 1, 2 and 3) and yellowness b^*, where NP fish were more yellow (significantly mostly in all measured locations) than AP fish (switched to a more blue colour), while almost no significant difference was detected in redness a^* (Table 3). We found that the ventral parts of all examined fillets were significantly lighter, more yellow and red than the dorsal ones that could be associated with the distribution of fat in fish muscle that is usually lower in the dorsal and posterior parts than in the ventral and anterior parts of fish fillet (Nortvedt & Tuene, 1998; Roth et al., 2007).

3.6 | Sensory evaluation

Only one significant difference ($p < 0.05$) among the four analysed groups was found in post-rigor cooked APF fillets in one of the five sensory attributes, the aftertaste, with a lower sensory score (8.21) indicating almost no aftertaste. The cooked AP fillets were assessed as better in all the five classified sensory parameters (Figure 7a); however, without significant differences as well as among the four experimental groups in post-rigor raw fillets for texture, colour, odour and overall acceptability. However, higher scores (=better sensory attributes) were evaluated for raw AP fillets (both sexes) in all tested parameters (Figure 7b). Therefore, cooked as well as raw AP fillets of both sexes were more acceptable for evaluators. These results indicate that extension of the light period of the day has a positive effect on a sensory evaluation of brook trout flesh, but the effect is only minimal.

3.7 | Fat content and FA profiles

Total lipid content, FA composition (% of identified), and nutritional indexes $n-3/n-6$, $n-6/n-3$ and P/S of four experimental groups were evaluated (Table 4). The lipid content was higher in APF (7.50% ± 0.78%) and APM (7.75% ± 1.16%) in comparison with NPF (6.32% ± 0.77%) and NPM (6.49% ± 1.26%); however, the only significant difference ($p < 0.05$) was detected between APM and

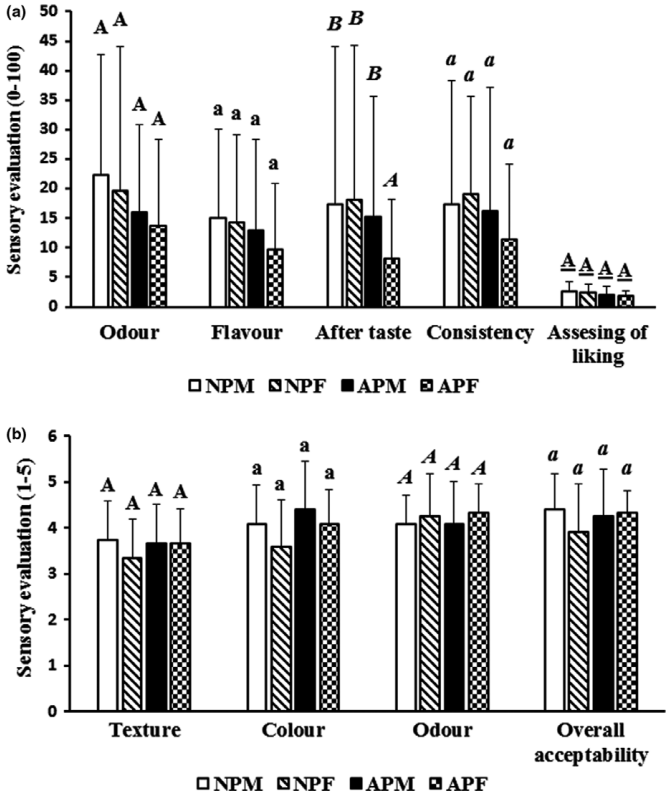

FIGURE 7 Sensory evaluation of post-rigor (A) cooked (the best 0–100 the worst) and (B) raw (the best 5–1 the worst) fillets of brook trout. Results are expressed as mean (bars) ± standard deviation (whiskers). Bars having the same character are not significantly different (hierarchical ANOVA, Fischer´s LSD test, $p > 0.05$), $n = 10$ per treatment

TABLE 4 Fatty acid composition, total lipid content (% of identified) and nutritional indexes $n-3/n-6$, $n-6/n-3$ and P/S of brook trout males/females treated under natural/artificial photoperiod

	Brook trout males (M)/females (F) under natural (NP)/artificial (AP) photoperiod (mean ± *SD*)			
Lipid and fatty acids (%)	**NPM**	**NPF**	**APM**	**APF**
Total lipid content	6.49 ± 1.26^{a,b}	6.32 ± 0.77^{a}	7.75 ± 1.16^{b}	7.50 ± 0.78^{a,b}
C14:0	1.66 ± 0.04^{a,b}	1.67 ± 0.11^{a}	1.80 ± 0.19^{a,b}	1.86 ± 0.11^{b}
C16:0	9.51 ± 0.32^{a,b}	8.89 ± 0.21^{a}	9.37 ± 0.75^{a,b}	9.69 ± 0.33^{b}
C16:1	3.24 ± 0.04^{a}	3.35 ± 0.17^{a,b}	3.64 ± 0.40^{a,b}	3.68 ± 0.28^{b}
C18:0	2.09 ± 0.09^{b}	1.89 ± 0.04^{a}	1.96 ± 0.05^{a}	1.94 ± 0.09^{a}
C18:1*n* − 9	43.19 ± 1.40^{a}	43.60 ± 0.71^{a}	43.71 ± 0.73^{a}	43.26 ± 0.62^{a}
C18:1*n* − 7	3.22 ± 0.06^{a}	3.36 ± 0.11^{a,b}	3.39 ± 0.04^{b}	3.48 ± 0.12^{b}
C18:2*n* − 6	15.41 ± 0.36^{a}	15.04 ± 0.21^{a}	15.17 ± 0.40^{a}	14.92 ± 0.36^{a}
C18:3*n* − 3	4.55 ± 0.13^{b}	4.27 ± 0.12^{a}	4.62 ± 0.14^{b}	4.51 ± 0.18^{b}
C20:0	0.21 ± 0.01^{a,b}	0.20 ± 0.02^{a}	0.23 ± 0.03^{b}	0.22 ± 0.01^{a,b}
C20:1*n* − 9	3.08 ± 0.10^{a}	3.73 ± 0.17^{c}	3.34 ± 0.30^{a,b}	3.40 ± 0.14^{b}
C20:2*n* − 6	1.15 ± 0.25^{a,b}	1.32 ± 0.10^{b}	1.01 ± 0.07^{a}	1.14 ± 0.09^{a}
C20:4*n* − 6	0.37 ± 0.10^{a,b}	0.41 ± 0.03^{b}	0.32 ± 0.05^{a}	0.37 ± 0.05^{a,b}
C20:3*n* − 3	0.30 ± 0.02^{a}	0.33 ± 0.02^{a}	0.30 ± 0.06^{a}	0.32 ± 0.03^{a}
C22:0	0.11 ± 0.02^{a,b}	0.08 ± 0.03^{a}	0.14 ± 0.06^{b}	0.11 ± 0.03^{a,b}
C22:1	0.96 ± 0.07^{a}	0.95 ± 0.13^{a}	0.97 ± 0.12^{a}	0.96 ± 0.07^{a}
C20:5*n* − 3	1.84 ± 0.28^{a}	1.94 ± 0.14^{a}	1.85 ± 0.18^{a}	2.00 ± 0.09^{a}
C24:1	0.30 ± 0.06^{a,b}	0.28 ± 0.02^{a}	0.36 ± 0.05^{b}	0.33 ± 0.05^{a,b}
C22:5*n* − 3	0.79 ± 0.13^{a}	0.96 ± 0.06^{b}	0.81 ± 0.12^{a}	0.87 ± 0.09^{a,b}
C22:6*n* − 3	8.02 ± 1.09^{a}	7.74 ± 0.74^{a}	7.02 ± 1.04^{a}	6.95 ± 0.40^{a}
ΣSFA	13.58 ± 0.42^{a,b}	12.72 ± 0.25^{a}	13.50 ± 0.86^{b}	13.82 ± 0.44^{b}
ΣMUFA	53.99 ± 1.63^{a}	55.27 ± 0.78^{a}	55.40 ± 0.74^{a}	55.11 ± 0.65^{a}
ΣPUFA	32.44 ± 1.24^{a}	32.02 ± 0.74^{a}	31.10 ± 1.30^{a}	31.07 ± 0.89^{a}
Σ*n* − 3 PUFA	15.50 ± 1.38^{a}	15.24 ± 0.82^{a}	14.59 ± 1.31^{a}	14.65 ± 0.60^{a}
Σ*n* − 6 PUFA	16.93 ± 0.25^{b}	16.78 ± 0.23^{a,b}	16.51 ± 0.35^{a,b}	16.42 ± 0.37^{a}
Σ*n* − 3 HUFA	10.95 ± 1.49^{a}	10.97 ± 0.84^{a}	9.97 ± 1.35^{a}	10.14 ± 0.49^{a}
EPA + DHA	9.87 ± 1.36^{a}	9.68 ± 0.81^{a}	8.87 ± 1.20^{a}	8.94 ± 0.44^{a}
n − 3/*n* − 6	0.92 ± 0.09^{a}	0.91 ± 0.06^{a}	0.88 ± 0.09^{a}	0.89 ± 0.03^{a}
n − 6/*n* − 3	1.10 ± 0.11^{a}	1.10 ± 0.07^{a}	1.14 ± 0.10^{a}	1.12 ± 0.04^{a}
P/S	2.39 ± 0.04^{a}	2.52 ± 0.07^{a}	2.32 ± 0.25^{a}	2.25 ± 0.13^{a}

Note. Data are shown as mean ± standard deviation (*SD*). Values followed by the same letters in the same row do not differ significantly (Tukey´s HSD test, $p > 0.05$), $n = 10$ per treatment.

APF: artificial photoperiod female; APM: artificial photoperiod male; NPF: natural photoperiod female; NPM: natural photoperiod male; DHA: docosahexaenoic fatty acid; EPA: eicosapentaenoic fatty acid; HUFA: highly unsaturated fatty acids; MUFA: monounsaturated fatty acids; $n-3/n-6$: ratio between Σ of the Omega 3/Σ of the Omega 6 polyunsaturated fatty acids; $n-6/n-3$: ratio between Σ of the Omega 6/Σ of the Omega 3 polyunsaturated fatty acids; P/S: polyunsaturated/saturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

NPF. According to Ackman (1990), fish in the present study were considered to be medium fat fish (5%–10%). Nineteen FA (%), composed of C14:0 to C22:6*n* − 3 that exceeded the minimum of 0.1% of the total FA in all samples, were identified (Table 4). Moreover, the sum of saturated FA (SFA), monounsaturated FA (MUFA), PUFA, HUFA with EPA, and DHA were calculated and followed the patterns: MUFA > PUFA > SFA and *n* − 6 PUFA > *n* − 3 PUFA.

The sum of all identified SFA ranged from 12.72% ± 0.25% to 13.82% ± 0.44% and was significantly higher ($p < 0.05$) in female from AP than NP. The palmitic acid (C16:0) was detected at the highest level and its relative content was significantly higher in APF than NPF. The myristic acid (C14:0) was present at low concentration (in all studied fish), demonstrating a positive factor for human health, because its high concentration supports the emergence of hypercholesterolemia (Fernandes et al., 2014). Monounsaturated FA constituted more than half of the total FA content (53.99% to 55.40%) with no significant differences among groups; oleic acid (C18:1*n* − 9) was the most abundant MUFA in all studied fish. Also,

the total PUFA level (31.07% to 32.44%) was observed with no significant differences. The $n-3/n-6$ ratio did not reach the value 1.0 showing to higher values of $n-6$ PUFA than $n-3$ PUFA. This result was expected because examined fish were fresh water species and not marine fish containing a higher proportion of $n-3$ PUFA (Fernandes et al., 2014). Freshwater fish usually have higher proportions of $n-6$ PUFA, especially linolenic acid (C18:2$n-6$) (Huynh & Kitts, 2009; Ozogul, Ozogul, & Alagoz, 2007) that is also reported in the present study. Nevertheless, DHA was the predominant (48% from $n-3$ PUFA) among the total amount of $n-3$ PUFA and exceeded the content of EPA in all studied fish. The $n-6/n-3$ ratio below 4.0 has been shown to prevent the risk of cardiovascular diseases (Department of Health & Social Security, Diet & cardiovascular disease, 1984) and this ratio ranged from 1.10 in NP to 1.14 AP fish. Also the P/S ratio was determined because it indicates the possibility to induce a cholesterol increase in the blood. The P/S value below 0.45 has been considered to be undesirable for the human diet (Department of Health & Social Security, Diet & cardiovascular disease, 1984) and did not reach this value in the present study (>2 in all experimental fish). Due to these nutritional indices, studied fish can be categorised as beneficial for human consumption. Our results indicate that photoperiod did not change the FA composition in large scale and is mainly influenced by the type of culture system especially by the diet in brook trout. This is in contradiction with the study of Manor et al. (2012), Manor et al. (2014), who reported that photoperiod affects the composition of FA in rainbow trout.

4 | CONCLUSION

This study evaluates the impact of photostimulation on post-mortem changes, sensory, textural and nutritional characteristics of flesh of brook trout reared in an intensive culture system in the Czech Republic. Lower values of pH were measured in fish under AP during the entire measurement with faster progress of rigor mortis in comparison to fish under NP, which was assumed to be vice versa and was probably affected by the length of starvation (only 48 hr in AP group) in comparison to non-feed intake in NP group. Significantly higher fillet contraction ($p < 0.05$) more than 7% were observed among AP females compared to the control groups. In gaping evaluation, AP groups exhibited a medium degree of gaping with softer texture compared to NPF with a significantly lower degree of gaping (mild). Also higher values of hardness were observed in control fish in comparison with AP fish. In colour measurement, significant differences were detected in lightness L^*, where NP fish (mature) were lighter than AP fish (immature) and yellowness b^*, with more yellow flesh in NP group. We documented that extension of the light period has a positive effect on the sensory evaluation of brook trout flesh, but the effect is only minimal. Finally, we indicated that the fat content and FA composition in brook trout flesh are not in a large scale affected by the photoperiod. These data are considered as preliminary results. Consequently, further experiments have to be done to resolve the contraindications documented in the present study.

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CONFLICT OF INTEREST

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REFERENCES

Ackman, R. G. (1990). Seafood lipids and fatty acids. *Food Reviews International*, *6*, 617–646. https://doi.org/10.1080/87559129009540896

Adamkova, V., Kacer, P., Mraz, J., Suchanek, P., Pickova, J., Kralova Lesna, I., … Maratka, V. (2011). The consumption of the carp meat and plasma lipids in secondary prevention in the heart ischemic disease patients. *Neuroendocrinology Letters*, *32*, 101–104.

Aksnes, A., Gjerde, B., & Roald, S. O. (1986). Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture*, *53*, 7–20. https://doi.org/10.1016/0044-8486(86)90295-4

Andersen, U. B., Stromsnes, A. N., Steinsholt, K., & Thomassen, M. S. (1994). Fillet gaping in farmed Atlantic salmon (*Salmo salar*). *Norwegian Journal of Agricultural Sciences*, *8*, 165–179.

Andersen, U. B., Thomassen, M. S., & Rørå, A. M. B. (1997). Texture properties of farmed rainbow trout (*Oncorhynchus mykiss*): Effects of diet, muscle fat content and time of storage on ice. *Journal of the Science of Food and Agriculture*, *74*, 347–353. https://doi.org/10.1002/(SICI)1097-0010(199707)74:3aabbb347:AID-JSFA802aaabb3.0.CO;2-F

Appelqvist, L. A. (1968). Rapid methods of lipid extraction and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing accumulation of lipid contaminants. *Arkiv För Kemi*, *28*, 551–570.

Berg, T., Erikson, U., & Nordtvedt, T. S. (1997). Rigor mortis assessment of Atlantic salmon (*Salmo salar*) and effects of stress. *Journal of Food Science*, *62*, 439–446. https://doi.org/10.1111/j.1365-2621.1997.tb04402.x

Bito, M. (1986). The influence of freshness of the fish, freezing temperature, thawing rate and thawing temperature on thaw rigor. *Bulletin of Tokai Regional Fisheries Research Laboratory*, *119*, 25–31.

Bromage, N. R., Elliot, J. A., Springate, J. R. C., & Whitehead, C. (1984). The effects of constant photoperiods on the timing of spawning in rainbow trout. *Aquaculture*, *43*, 213–223.

Brouwer, I. A., Geelen, A., & Katan, M. B. (2006). n-3 fatty acids, cardiac arrhythmia and fatal coronary heart disease. *Progress in Lipid Research*, *45*, 357–367. https://doi.org/10.1016/j.plipres.2006.02.004

Bruno, D. W. (1989). Fungal infections of farmed salmon and trout. *Aquaculture Information S.*, *5*, 1–5.

Cleveland, B. M., Kenney, P. B., Manor, M. L., & Weber, G. M. (2012). Effects of feeding level and sexual maturation on carcass and fillet characteristics and indices of protein degradation in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *338–341*, 228–236. https://doi.org/10.1016/j.aquaculture.2012.01.032

Currie, J., & Woo, P. (2007). Susceptibility of sexually mature rainbow trout, *Oncorhynchus mykiss* to experimental cryptobiosis caused by *Cryptobia salmositica*. *Parasitology Research*, *101*, 1057–1067. https://doi.org/10.1007/s00436-007-0586-8

Department of Health and Social Security, Diet and cardiovascular disease. (1984). *Report on health and social subjects*, pp. 443–456. London, UK: HMSO.

Duncan, N. J., Mitchell, D., & Bromage, N. R. (1999). Post-smolt growth and maturation of out-of-season 0+ Atlantic salmon (*Salmo salar*) reared under different photoperiods. *Aquaculture*, *177*, 61–77. https://doi.org/10.1016/S0044-8486(99)00069-1

Einen, O., Mørkøre, T., Rørå, A. M. B., & Thomassen, M. S. (1999). Feed ration prior to slaughter - A potential tool for managing product quality of Atlantic salmon (*Salmo salar*). *Aquaculture*, *178*, 149–169. https://doi.org/10.1016/S0044-8486(99)00126-X

Einen, O., & Thomassen, M. S. (1998). Starvation prior to slaughter in Atlantic salmon (*Salmo salar*): II. White muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. *Aquaculture*, *169*, 37–53. https://doi.org/10.1016/S0044-8486(98)00332-9

Endal, H. P., Taranger, G. L., Stefansson, S. O., & Hansen, T. (2000). Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. *Aquaculture*, *191*, 337–349. https://doi.org/10.1016/S0044-8486(00)00444-0

Eriksson, S. F., & Pickova, J. (2007). Fatty acids and tocopherol levels in *M. Longissimus dorsi* of beef cattle in Sweden - A comparison between seasonal diets. *Meat Science*, *76*, 746–754. https://doi.org/10.1016/j.meatsci.2007.02.021

Espe, M., Ruohonen, K., Bjornevik, M., Froyland, L., Nortvedt, R., & Kiessling, A. (2004). Interactions between ice storage time, collagen composition, gaping and textural properties in farmed salmon muscle harvested at different times of the year. *Aquaculture*, *240*, 489–504. https://doi.org/10.1016/j.aquaculture.2004.04.023

Fernandes, C. E., Vasconcelos, M. A., Ribeiro Mde, A., Sarubbo, L. A., Andrade, S. A., & Filho, A. B. (2014). Nutritional and lipid profiles in marine fish species from Brazil. *Food Chemistry*, *160*, 67–71. https://doi.org/10.1016/j.foodchem.2014.03.055

Fletcher, G. C., Hallett, A. R., & Holland, A. J. (1997). Changes in the fine structure of the myocommata-muscle fibre junction related to gaping in rested an exercised muscle from king salmon (*Oncorhynchus tshawytscha*). *LWT – Food Science and Technology*, *30*, 246–252.

Foss, A., Imsland, A. K., Vikingstad, E., Stefansson, A. O., Norberg, B., Pedersen, S., … Roth, B. (2009). Compensatory growth in Atlantic halibut: Effect of starvation and subsequent feeding on growth, maturation, feed utilization and flesh quality. *Aquaculture*, *290*, 304–310. https://doi.org/10.1016/j.aquaculture.2009.02.021

Gorgun, S., & Akpinar, M. A. (2007). Liver and muscle fatty acid composition of mature and immature rainbow trout (*Oncorhynchus mykiss*) fed two different diets. *Biologia Bratislava*, *62*, 351–355. https://doi.org/10.2478/s11756-007-0058-8

Hagen, Ø., & Johnsen, C. A. (2016). Flesh quality and biochemistry of light-manipulated Atlantic cod (*Gadus morhua*) and the significance of collagen cross-links on fillet firmness and gaping. *Food Chemistry*, *190*, 786–792. https://doi.org/10.1016/j.foodchem.2015.06.007

Hansen, T., Karlsen, Ø., Taranger, G. L., Hemre, G. I., Holm, J. C., & Kjesbu, O. S. (2001). Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture*, *203*, 51–67. https://doi.org/10.1016/S0044-8486(01)00610-X

Hansen, T., Stefansson, S. O., & Taranger, G. L. (1992). Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. *Aquaculture and Fisheries Management*, *23*, 275–280.

Hara, A., & Radin, N. S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry*, *90*, 420–426. https://doi.org/10.1016/0003-2697(78)90046-5

Henderson, N. E. (1963). Influence of light and temperature on the reproductive cycle of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). *Journal of the Fisheries Research Board of Canada*, *20*, 859–897.

Holcombe, G. W., Pasha, M. S., Jensen, K. M., Tietge, J. E., & Ankle, G. T. (2000). Effects of photoperiod manipulation on brook trout reproductive development, fecundity, and circulating sex steroid concentrations. *North American Journal of Aquaculture*, *62*, 1–11.

Huynh, M. D., & Kitts, D. D. (2009). Evaluating nutritional quality of pacific fish species from fatty acid signatures. *Food Chemistry*, *114*, 912–918. https://doi.org/10.1016/j.foodchem.2008.10.038

Johnston, I. A., Alderson, R., Sandham, C., Dingwall, A., Mitchell, D., Selkirk, C., … Springate, J. (2000). Muscle fibre density in relation to colour and texture of smoked Atlantic salmon (*Salmo salar* L.). *Aquaculture*, *189*, 335–349. https://doi.org/10.1016/s0044-8486(00)00373-2

Johnston, C. E., Hambrook, M. J., Gray, R. W., & Davidson, K. G. (1992). Manipulation of reproductive function in Atlantic salmon (*Salmo salar*) kelts with controlled photoperiod and temperature. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*, 2055–2061. https://doi.org/10.1139/f92-228

Johnston, I. A., Manthri, S., Alderson, R., Smart, A., Campbell, P., Mitchell, D., … Robertson, B. (2002). Effects of dietary protein level on muscle cellularity and flesh quality in Atlantic salmon with particular reference to gaping. *Aquaculture*, *210*, 259–283. https://doi.org/10.1016/S0044-8486(01)00862-6

Johnston, I. A., Manthri, S., Bickerdike, R., Dingwall, A., Luijkx, R., Campbell, P., … Alderson, R. (2004). Growth performance, muscle structure and flesh quality in out-of-season Atlantic salmon (*Salmo salar*) smolts reared under two different photoperiod regimes. *Aquaculture*, *237*, 281–300. https://doi.org/10.1016/j.aquaculture.2004.04.026

Kris-Etherton, P. M., Harris, W. S., Appel, L. J., Association, A. H., & Committee, N. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, *106*, 2747–2757. https://doi.org/10.1161/01.CIR.0000038493.65177.94

Kristoffersen, S., Tobiassen, T., Steinsund, V., & Olsen, R. L. (2006). Slaughter stress, post mortem muscle pH and rigor development in farmed Atlantic cod (*Gadus morhua* L.). *International Journal of Food Science and Technology*, *41*, 861–864. https://doi.org/10.1111/j.1365-2621.2005.01149.x

Kristoffersen, S., Vang, B., Larsen, R., & Olsen, R. L. (2007). Pre-rigor filleting and drip loss from fillets of farmed Atlantic cod (*Gadus morhua* L.). *Aquaculture Research*, *38*, 1556–1564. https://doi.org/10.1111/j.1365-2109.2007.01843.x

Lavety, J., Afolabi, O. A., & Love, R. M. (1988). The connective tissues of fish. Gaping in farmed species. *International Journal of Food Science and Technology*, *23*, 23–30.

Leclercq, E., Taylor, J. F., Hunter, D., & Migaud, H. (2010). Body size dimorphism of sea-reared Atlantic salmon (*Salmo salar* L.): Implications for the management of sexual maturation and harvest quality. *Aquaculture*, *301*, 47–56. https://doi.org/10.1016/j.aquaculture.2010.01.029

Lund, E. K. (2013). Health benefits of seafood; Is it just the fatty acids? *Food Chemistry*, *140*, 413–420. https://doi.org/10.1016/j.foodchem.2013.01.034

Manor, M. L., Weber, G. M., Cleveland, B. M., & Kenney, P. B. (2014). Effects of feeding level and sexual maturation on fatty acid composition of energy stores in diploid and triploid rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *418–419*, 17–25. https://doi.org/10.1016/j.aquaculture.2013.09.023

Manor, M. L., Weber, G. M., Salem, M., Yao, J., Aussanasuwannakul, A., & Kenney, P. B. (2012). Effect of sexual maturation and triploidy on chemical composition and fatty acid content of energy stores in female rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *364–365*, 312–321. https://doi.org/10.1016/j.aquaculture.2012.08.012

Másílko, J., Zajíc, T., & Hlaváč, D. (2015). The culture system affects organoleptic properties and lipid composition of common carp (*Cyprinus carpio* L.) meat. *Journal of Texture Studies*, *46*, 345–352. https://doi.org/10.1111/jtxs.12134

McCormick, S. D., & Naiman, R. J. (1984). Some determinants of maturation in brook trout (*Salvelinus fontinalis*). *Aquaculture*, *43*, 269–278. https://doi.org/10.1016/0044-8486(84)90028-0

Michie, I. (2001). Causes of downgrading in the salmon industry. In S. C. Kestin, & P. D. Warriss (Eds.), *Farmed fish quality* (pp. 129–136). Oxford: Blackwell Science.

Mørkøre, T., Hansen, S. J., & Rørvik, K. A. (2006). Relevance of storage temperature for contraction and gaping of pre-rigor filleted farmed cod (*Gadus Morhua* L.). In J. B. Luten, C. Jacobsen, K. Bekaert, A. Saebo, & J. Oehlenschlager (Eds.), *Seafood research from fish to dish: Quality, safety and processing of wild and farmed fish* (pp. 173–184). Wageningen, the Netherlands: Wageningen Academic Publishers.

Mørkøre, T., Mazo, T., Tahirovic, V., & Einen, O. (2008). Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (*Salmon salar* L). *Aquaculture*, *277*, 231–238. https://doi.org/10.1016/j.aquaculture.2008.02.036

Mraz, J., & Pickova, J. (2009). Differences between lipid content and composition of different parts of fillets from crossbred farmed carp (*Cyprinus carpio*). *Fish Physiology and Biochemistry*, *35*, 615–623. https://doi.org/10.1007/s10695-008-9291-5

Nortvedt, R., & Tuene, S. (1998). Body composition and sensory assessment of three weight groups of Atlantic halibut (*Hippoglossus hippoglossus*) fed three pellet sizes and three dietary fat levels. *Aquaculture*, *161*, 295–313. https://doi.org/10.1016/S0044-8486(97)00277-9

Ozogul, Y., Ozogul, F., & Alagoz, S. (2007). Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: A comparative study. *Food Chemistry*, *103*, 217–223. https://doi.org/10.1016/j.foodchem.2006.08.009

Paaver, T., Gross, R., & Ilves, P. (2004). Growth rate, maturation level and flesh quality of three strains of large rainbow trout (*Oncorhynchus mykiss*) reared in Estonia. *Aquaculture International*, *12*, 33–45. https://doi.org/10.1023/B:AQUI.0000017185.10472.1d

Reid, R. A., & Durance, T. D. (1992). Textural changes of canned chum salmon related to sexual maturity. *Journal of Food Science*, *57*, 1340–1342. https://doi.org/10.1111/j.1365-2621.1992.tb06852.x

Roth, B., Jenssen, M. D., Jonassen, T. M., Foss, A., & Imsland, A. (2007). Change in flesh quality associated with early maturation of Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture Research*, *38*, 757–763. https://doi.org/10.1111/j.1365-2109.2007.01729.x

Roth, B., Johansen, S. J. S., Suontama, J., Kiessling, A., Leknes, O., Guldberg, B., & Handeland, S. (2005). Seasonal variation in flesh quality, comparison between large and small Atlantic salmon (*Salmo salar*) transferred into seawater as 0+ or 1+ smolts. *Aquaculture*, *250*, 830–840. https://doi.org/10.1016/j.aquaculture.2005.05.009

Salem, M., Kenney, P. B., Rexroad, C. E., & Yao, J. (2006). Molecular characterization of muscle atrophy and proteolysis associated with spawning in rainbow trout. *Comparative Biochemistry and Physiology*, *1*, 227–237. https://doi.org/10.1016/j.cbd.2005.12.003

Salem, M., Silverstein, J., Rexroad, C. E., & Yao, J. (2007). Effect of starvation on global gene expression and proteolysis in rainbow trout (*Oncorhynchus mykiss*). *BMC Genomics*, *8*, 328–343. https://doi.org/10.1186/1471-2164-8-328

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy*, *56*, 365–379. https://doi.org/10.1016/S0753-3322(02)00253-6

Smith, I. P., Metcalfe, N. B., Huntingford, F. A., & Kadri, S. (1993). Daily and seasonal patterns in the feeding behaviour of Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture*, *117*, 165–178. https://doi.org/10.1016/0044-8486(93)90133-J

Stejskal, V., Vejsada, P., Cepak, M., Špička, J., Vacha, F., Kouril, J., & Policar, T. (2011). Sensory and textural attributes and fatty acids profiles of fillets of extensively and intensively farmed Eurasian perch (*Perca fluviatilis* L.). *Food Chemistry*, *129*, 1054–1059. https://doi.org/10.1016/j.foodchem.2011.05.073

Takashima, F., & Yamada, Y. (1984). Control of maturation in masu salmon by manipulation of photoperiod. *Aquaculture*, *43*, 243–257. https://doi.org/10.1016/0044-8486(84)90026-7

Taranger, G. L., Carrillo, M., Schulz, R. W., Fontaine, P., Zanuy, S., Felip, A., … Hansen, T. (2010). Control of puberty in farmed fish. *General and Comparative Endocrinology*, *165*, 483–515. https://doi.org/10.1016/j.ygcen.2009.05.004

Taranger, G. L., Haux, C., Hansen, T., Stefansson, S. O., Björnsson, B. T., Walter, B. T., & Kryvi, H. (1999). Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. *Aquaculture*, *177*, 47–60. https://doi.org/10.1016/S0044-8486(99)00068-X

Taranger, G. L., Haux, C., Steffansson, S. O., Björnsson, B. T., Walther, B. T., & Hansen, T. (1998). Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17β profiles in Atlantic salmon, *Salmo salar*. *Aquaculture*, *162*, 85–98. https://doi.org/10.1016/S0044-8486(98)00168-9

Traxler, G. S., Roome, J. R., Lauda, K. A., & LaPatra, S. (1997). Appearance of infectious haematopoietic necrosis virus (IHNV) and neutralizing antibodies in sockeye salmon *Oncorhynchus nerka* during their migration and maturation period. *Diseases of Aquatic Organisms*, *28*, 31–38. https://doi.org/10.3354/dao028031

Varga, D., Szabo, A., Hancz, C., Jeney, Z., Ardo, L., Molnar, M., & Molnar, T. (2014). Impact of handling and pre-mortal stress on the flesh quality of common carp (*Cyprinus carpio* L.). *Israeli Journal of Aquaculture - Bamidgeh*, *66*, 1–6.

Veland, J. O., & Torrissen, O. J. (1999). The texture of Atlantic salmon (*Salmo salar*) muscle as measured instrumentally using TPA and Warner-Brazler shear test. *Journal of the Science of Food and Agriculture*, *79*, 1737–1746. https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1737:AID-JSFA432>3.0.CO;2-Y

Whitehead, C., & Bromage, N. (1980). Effects of constant long and short day photoperiods on the reproductive physiology and spawning of the rainbow trout. *Journal of Endocrinology*, *87*, 6–7.

Wood, J. D., Richarson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., … Enser, M. (2003). Effects of fatty acids on meat quality: A review. *Meat Science*, *66*, 21–32. https://doi.org/10.1016/S0309-1740(03)00022-6

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CHAPTER 6

General discussion

Fish are guided by signals from the external environment to achieve synchronization of maturation processes with the changing of seasons. Even though photoperiod, temperature, rainfall, pheromones and food supplies – are approximate, it is assumed that the seasonally changing pattern of photoperiod is probably responsible for the development of the reproduction process in the majority of fish species. This statement is fully consistent with the principle of maturation in the salmonids (Bromage et al., 2001). During salmonids reproduction, one can observe an energy shift from somatic growth to maintain reproductive ability and produce a massive number of oocytes — which are filled with a large amount of vitellogenin (Kause et al., 2003; Noori et al., 2015). In summarizing the numerous scientific data, we can conclude that sexual maturation in salmonids affects their growth, feeding performance, health conditions and flesh quality (Linhartová et al., 2018; McMillan et al., 2012; Sacobie et al., 2016). From an economical point of view, this phenomenon means a higher cost of feed and a lower profitability for salmonid enterprises (Noori et al., 2015).

Considering the growing interest in inhibiting or reducing the incidence of fish maturity, numerous studies have been conducted on photoperiod manipulation (acceleration, advancement, continuous illumination or discrete photophases). Currently, it has been confirmed that the photoperiod stimulated endogenous physiological rhythms, growth rate, regulated appetite, and also synchronised sexual maturation and reproduction in fish (Taranger et al., 2010). The fish reactions to photoperiod manipulation are dependent on their physiological condition, nutritional state and timing of these manipulations (Hansen et al., 2001). At the moment, it has been proven that in many cultivated species, including salmonids, artificial prolonged photoperiods can be used for delaying their sexual maturation (Skilbrei and Heino, 2011; Taylor et al., 2006; Unwin et al., 2005).

For testing the influence of artificially prolonged photoperiod on sexual maturation and growth rate in brook trout, we used three different approaches, namely the application of various light sources (viz. light-emitting diodes and metal-halide lights) (Chapter 2), timing of the photoperiod extension at different days for each group (Chapter 3), and analysing the effect of various non-circadian photoperiod regimes on the growth performance and puberty of brook trout (Chapter 4).

Based on the results of Chapters 2, 3, 4, the use of artificially prolonged photoperiod regimes can be recommended for delaying sexual maturation in brook trout. The Chapter 5 is a continuation of the investigations described in Chapter 3. In this regard, all further description of the duplicated data will be indicated as in Chapter 3. In all experiments, control groups that were reared under natural ambient photoperiod conditions showed the highest level of preparedness for spawning — regardless of sex. According to the results of our testing of different light sources (Chapter 2), there were no significant differences between the experimental groups exposed to an extended photoperiod using different light sources. However, after an economic evaluation, we can recommend light-emitting diodes for use in practice, since this light source is more cost-effective and produces the same positive influence on other important physiological parameters. During the study of various timing regimes of the prolonged photoperiod, three experimental (each group had its own timing strategy) and control treatments were tested (Chapter 3). The results indicate that the most acceptable strategy was to start using artificially prolonged lighting with the time range from 19 September (day 88 post-midsummer) to 24 November, which is 66 days of artificially extended photoperiod with a light regime of 18L:6D. This period proved to be profitable and sufficient to obtain the intended positive results. In Chapter 4, the effect of three different non-circadian photoperiod regimes on brook trout was compared with the influence of natural

ambient light conditions. Based on the results, the optimal condition for effective use of non-circadian regime is using the strategy of continuous daylight (24 h) for one day followed by two days of natural ambient light photoperiod. This experimental treatment presented better results in the delaying of maturity (there were no individuals prepared for spawning when fish in the control group were ready to spawn) and significant higher somatic growth rates in contrast to control groups. It should be noted, that in all experiments a significantly larger number of mature individuals were males. This finding confirms the general concept that males often mature earlier than females (Imsland et al., 1997a; Imsland et al., 1997b; Taranger et al., 2010). The obtained data on readiness for spawning are consistent with previous studies in salmonid species (Hansen et al., 2017; Noori et al., 2015; Taranger et al., 1998).

Sex steroids are some of the indicators of level of maturity and they play a significant role in the regulation of vitellogenin synthesis and the proliferation of spermatogonia during spermatogenesis (Qiu et al., 2015; Schulz et al., 2010). At all stages of the experiments carried out during this thesis, there was a similar tendency to increase the level of sex steroids in the control groups (Chapters 2, 3, 4). In each experiment, a significant increase in oestradiol and testosterone was detected in fish reared under natural ambient light conditions. The rise in oestradiol level in females was in accordance with the work of Tam et al. (1986), who suggested that the highest level of oestradiol in female brook trout is observed near the end of vitellogenesis. In our study, the overall picture obtained of the state of oestradiol and its changes is consistent with previous studies (Frantzen et al., 2004; Mayer et al., 1990; Pavlidis et al., 1994). Analyses of testosterone level and its changes in males are in agreement with reported results for brook trout and other salmonids (Holcombe et al., 2000; Qiu et al., 2015). One of the main points in the analyses of plasma sex steroids was the discovery of a similar increase in steroid levels in other experimental groups exposed to any kind of artificially extended photoperiod regime, after a certain period of time. This observation may indicate a process of delayed puberty in brook trout due to the influence of prolonged photoperiod regimes. As further confirmation of the above statement, an analysis of the gonadosomatic index development can be used. GSI changes, typical for the spawning period, were pronounced in all experiments that determined the effect of prolonged light regimes on the maturity of brook trout (Chapter 2, 3, 4). All control groups showed a significantly higher GSI index during the natural spawning time for brook trout compared to experimental treatments, regardless of gender. In all three experiments, males peaked at the GSI earlier than females as described above (Imsland et al., 2014; Taranger et al., 2010).

In the case of salmonids, photoperiod is perceived as a "zeitgeber" growth controlling factor through its influence on endogenous rhythms (Endal et al., 2000) or directly through photo-stimulation of the somatotropic axis (Björnsson, 1997; Taylor et al., 2006). During the implementation of this research, a similar tendency of growth-promoting effect by prolonged photoperiod was identified in all experiments, regardless of gender (Chapters 2, 3, 4). Fish from the control groups presented a significantly lower growth rate than individuals reared under artificial prolonged light regimes. The obtained results are in agreement with previously published works on this topic (Hansen et al., 2017; Karlsen et al., 2006; Liu and Duston, 2018; Önder et al., 2016). Analysing the available data, it can be assumed that growth increase in experimental treatments can be explained by a suppression of gonad development and a delay of the spawning process. This effect is most likely caused by the relocation of energy from gonad development to somatic growth – with a delay of puberty (Ginés et al., 2003; Noori et al., 2015). It is important to note that the growth stimulation effect in Chapters 2, 3 and 4 was not obtained by increasing the feeding period or feeding rate. We hypothesized that an increase of daylight hours led to this positive effect of the prolonged photoperiod on somatic growth rate. Randall et al. (2001) and Taylor et al. (2006) presented similar results. Their

works indicated an increase in growth without the use of additional feeding. Some previous studies presented conclusions about the negative effect of non-circadian photoperiods on the growth and survival of certain organisms (Dalley, 1979; Von Saint and Aschoff, 1978), but in our case, the best indicator of growth rate was demonstrated by experimental treatments, which were influenced by various types of non-circadian photoperiod regimes (Chapter 4).

In the experiment described in Chapter 2, the "Fish Skin Colour Evaluation Application" software was used to analyse the skin colouration of brook trout reared under prolonged photoperiod regimes with different light sources. The obtained results showed a difference in hue distribution between treatments reared under manipulated photoperiod and control groups (in both sexes). It was also recorded that males and females reared under natural ambient light conditions had more intense spawning colouration compared with other groups. However, for a more detailed study of this issue, further studies are needed. In parallel with this trial, a comparative analysis of the fins was performed (Chapter 2). At the end of the experiment, minimal fins injury was recorded, with higher relative fin length in the control groups.

Sacobie et al. (2016) reported that sexual maturation also can be characterized by increased susceptibility to disease and aggression of males (Currie and Woo, 2007). In our opinion, this is a rather important issue within the framework of intensive aquaculture. We conducted an additional short-term experiment in order to study the effect of continued photoperiod regimes on stress resistance and perception of diseases in brook trout (Chapter 2) after transport. The results obtained on survival and disease incidence in a presumably stressful situation (transportation) are presented in Chapter 2. The highest incidence of fungus-infected fish was found in the male control group reared under natural ambient light conditions. Most likely, this was provoked by increased male aggressiveness caused not only by sexual maturation, but also the influence of the stress factor from outside – changes in environmental conditions (transportation of live fish simulating common aquaculture practice for brook trout). It should be noted that in the analysis of survival rate, in this experiment the highest level of mortality was recorded in the control groups, regardless of gender. In general, we can say that these results correspond to the findings of Pickering and Duston (1983), which described the role of stress in the development of fungal disease in brown trout (*Salmo trutta* L.).

In the course of the second and third experiments (Chapter 3 and Chapter 4, respectively), an analysis of fillet yield was carried out. In both cases, a significantly lower fillet yield was obtained in the female control groups throughout the experimental period (Chapter 4) or during the first part of trial (Chapter 3), in contrast to the experimental groups, which were subject to a prolonged photoperiod.

In addition to the previous data in the framework of this study, an experiment describing the impact of photo-stimulation on the flesh quality of brook trout stored under refrigerated conditions was conducted (Chapter 5). It is well-known that puberty affects the flesh quality of other farmed salmonids. Namely, in the form of changing the body morphology, muscle lipid content, aftertaste, texture and colour of the flesh, for example, in rainbow trout (Cleveland et al., 2012; Görgün and Akpinar, 2007; Manor et al., 2014) and Atlantic salmon (Aksnes et al., 1986; Michie, 2001). Thus, sexual maturation not only changes the flesh quality, but also directly leads to a significant loss of market quality of products (Hines et al., 2019; Michie, 2001; Reid and Durance, 1992). Chapter 5 describes a study on the determination of post-mortem changes in brook trout made in experimental fish after the end of the main testing of various timing regimes of artificially prolonged photoperiod (Chapter 3). Aside from the standard parameters, indicators such as rigor index, pH changes, fillet contraction, gaping, flesh water losses, textural, colour, final fat content and fatty acid composition were analysed.

In the framework of this testing, sensory analyses were also carried out. The results of these tests showed that lower pH and faster flow of rigor were detected in the experimental group reared under artificial prolonged light regime. These data are in line with the study of Mørkøre et. al. (2008), who tested similar indicators in Atlantic salmon. A significantly higher (< 0.05) fillet contraction was found in the experimental female group (more than 7%). Hypothetically it can be assumed that this is due to a faster course of rigor mortis (Mørkøre et al., 2008). Based on a comparison of our results with published data, it be can concluded that the relative contraction did not contradict the range that was described for other fish species in previous works (Mørkøre et al., 2006, 2008). Furthermore, during testing it was determined that the prolonged photoperiod regime has a positive effect on the fat content, fatty acid composition, and sensory qualities. However, the effect in this experiment was only minimal, and further studies should be conducted to clarify the data already obtained and resolve all contraindications.

Compared with other major salmonid species, less attention has been given to the control of maturation by photoperiod manipulation in brook trout (Fatima, 2014) — and the reasons for this are unknown. However, as the results of the presented thesis show, the development of this topic can become promising and significantly beneficial for intensive aquaculture as a whole. These results can be used for the manipulation of the maturation and reproduction cycle to produce year-round eggs and fry, maintaining the general condition of the fish and preserving flesh quality. Thus the general appearance of the fish will increase the profitability of retail markets.

Conclusion

- An optimally ensured artificially prolonged photoperiod effectively affects the reproductive system in brook trout with a subsequent delay in sexual maturation, regardless of sex (Chapters 2, 3, 4).
- The use of extended light regimes has a positive effect on somatic growth, regardless of the sex of the fish (Chapters 2, 3, 4).
- In a frame of the study of the effect of a prolonged photoperiod and various light sources on the sexual maturation and overall performance in brook trout, light-emitting diodes (LED) are recommended in terms of economic benefits (Chapter 2).
- There were no significant differences in the skin colouration of brook trout under prolonged photoperiod when different light sources used, except for the presence of characteristic strong spawning colouration in control group reared under ambient natural light conditions (Chapter 2).
- A higher survival rate in the context of a stressful situation (environmental changes, transportation, susceptibility to fungal diseases) is ensured in brook trout reared under extended photoperiod (Chapter 2).
- Based on the data obtained, it is possible to recommend the use of a prolonged photoperiod to increase fish resistance against stress and fungal diseases (Chapter 2).
- The use of artificially prolonged light regimes can cause an improvement in the sensory qualities in brook trout, which in turn will increase the profitability of the market (Chapter 5).

References

Aksnes, A., Gjerde, B., Roald, S.O., 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. Aquaculture 53, 7–20.

Björnsson, B.T., 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiology and Biochemistry 17, 9–24.

Bromage, N., Porterm, M., Randallm, C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197, 63–98.

Cleveland, B.M., Kenney, P.B., Manor, M.L., Weber, G.M., 2012. Effects of feeding level and sexual maturation on carcass and fillet characteristics and indices of protein degradation in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 338, 228–236.

Currie, J.L., Woo, P.T., 2007. Susceptibility of sexually mature rainbow trout, *Oncorhynchus mykiss* to experimental cryptobiosis caused by *Cryptobia salmositica*. Parasitology Research 101, 1057–1067.

Dalley, R., 1979. Effects of non-circadian light cycles on the survival and development of *Palaemon elegans* Rathke reared in the laboratory, Cyclic Phenomena in Marine Plants and Animals. Elsevier, pp. 157–163.

Endal, H.P., Taranger, G.L., Stefansson, S.O., Hansen, T., 2000. Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. Aquaculture 191, 337–349.

Fatima, S., 2014. Reproductive biology and maturation control of brook trout (*Salvelinus fontinalis*, Mitchill) in Tasmania, National Centre for Marine Conservation and Resource Sustainability, Australian Maritime College, University of Tasmania. University of Tasmania, pp. 263.

Frantzen, M., Arnesen, A.M., Damsgård, B., Tveiten, H., Johnsen, H.K., 2004. Effects of photoperiod on sex steroids and gonad maturation in Arctic charr. Aquaculture 240, 561–574.

Ginés, R., Afonso, J., Argüello, A., Zamorano, M., Lopez, J., 2003. Growth in adult gilthead sea bream (*Sparus aurata* L.) as a result of interference in sexual maturation by different photoperiod regimes. Aquaculture Research 34, 73–83.

Görgün, S., Akpinar, M.A., 2007. Liver and muscle fatty acid composition of mature and immature rainbow trout (*Oncorhynchus mykiss*) fed two different diets. Biologia 62, 351–355.

Hansen, T., Karlsen, Ø., Taranger, G.L., Hemre, G.-I., Holm, J.C., Kjesbu, O.S., 2001. Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. Aquaculture 203, 51–67.

Hansen, T.J., Fjelldal, P.G., Folkedal, O., Vågseth, T., Oppedal, F., 2017. Effects of light source and intensity on sexual maturation, growth and swimming behaviour of Atlantic salmon in sea cages. Aquaculture Environment Interactions 9, 193–204.

Hines, C.W., Fang, Y., Chan, V.K., Stiller, K.T., Brauner, C.J., Richards, J.G., 2019. The effect of salinity and photoperiod on thermal tolerance of Atlantic and coho salmon reared from smolt to adult in recirculating aquaculture systems. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 230, 1–6.

Holcombe, G., Pasha, M., Jensen, K., Tietge, J., Ankley, G., 2000. Effects of photoperiod manipulation on brook trout reproductive development, fecundity, and circulating sex steroid concentrations. North American Journal of Aquaculture 62, 1–11.

Imsland, A., Folkvord, A., Grung, G., Stefansson, S., Taranger, G., 1997a. Sexual dimorphism in growth and maturation of turbot, *Scophthalmus maximus* (Rafinesque, 1810). Aquaculture Research 28, 101–114.

Imsland, A.K., Folkvord, A., Jónsdóttir, Ó.D., Stefansson, S.O., 1997b. Effects of exposure to extended photoperiods during the first winter on long-term growth and age at first maturity in turbot (*Scophthalmus maximus*). Aquaculture 159, 125–141.

Imsland, A.K., Handeland, S.O., Stefansson, S.O., 2014. Photoperiod and temperature effects on growth and maturation of pre-and post-smolt Atlantic salmon. Aquaculture International 22, 1331–1345.

Karlsen, Ø., Norberg, B., Kjesbu, O., Taranger, G., 2006. Effects of photoperiod and exercise on growth, liver size, and age at puberty in farmed Atlantic cod (*Gadus morhua* L.). ICES Journal of Marine Science 63, 355–364.

Kause, A., Ritola, O., Paananen, T., Mäntysaari, E., Eskelinen, U., 2003. Selection against early maturity in large rainbow trout *Oncorhynchus mykiss*: the quantitative genetics of sexual dimorphism and genotype-by-environment interactions. Aquaculture 228, 53–68.

Linhartová, Z., Lunda, R., Másílko, J., Dvořák, P., Lundová, K., Stejskal, V., Matoušek, J., Mráz, J., 2018. Impact of photostimulation for delayed maturity on flesh quality of brook trout (*Salvelinus fontinalis*) stored under refrigerated conditions. Aquaculture Research 49, 3817–3829.

Liu, Q., Duston, J., 2018. Efficacy of 24 h light to reduce maturation in Arctic charr (*Salvelinus alpinus*) is dependent on both the start date and duration. Aquaculture 484, 44–50.

McMillan, J.R., Dunham, J.B., Reeves, G.H., Mill, J.S., Jordan, C.E., 2012. Individual condition and stream temperature influence early maturation of rainbow trout and steelhead trout, *Oncorhynchus mykiss*. Environmental Biology of Fishes 93, 343–355.

Manor, M.L., Weber, G.M., Cleveland, B.M., Kenney, P.B., 2014. Effects of feeding level and sexual maturation on fatty acid composition of energy stores in diploid and triploid rainbow trout (*Oncorhynchus mykiss*). Aquaculture 418, 17–25.

Mayer, I., Lundqvist, H., Berglund, I., Schmitz, M., Schulz, R., Borg, B., 1990. Seasonal endocrine changes in Baltic salmon, *Salmo salar*, immature parr and mature male parr. I. Plasma levels of five androgens, 17α-hydroxy-20β-dihydroprogesterone, and 17βestradiol. Canadian Journal of Zoology 68, 1360–1365.

Michie, I., 2001. Causes of downgrading in the salmon farming industry. In: Kestin, S.C., Warriss, P.D. (Eds), Farmed Fish Quality, pp. 129–136.

Mørkøre, T., Hansen, S.J., Rørvik, K.-A., 2006. Relevance of storage temperature for contraction and gaping of pre rigor filleted farmed cod (*Gadus morhua* L.). In: Luten, J.B., Jacobsen, C., Bekaert, K., Saebo, A., Oehlenschlager, J. (Eds), Seafood research from fish to dish: Quality, safety and processing of wild and farmed fish. Wageningen Academic Publishers, the Netherlands, Wageningen, pp. 173–184.

Mørkøre, T., Tahirovic, V., Einen, O., 2008. Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (*Salmon salar* L). Aquaculture 277, 231–238.

Noori, A., Mojazi Amiri, B., Mirvaghefi, A., Rafiee, G., Kalvani Neitali, B., 2015. Enhanced growth and retarded gonadal development of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum) following a long-day photoperiod. Aquaculture Research 46, 2398–2406.

Önder, M.Y., Başçınar, N., Khan, U., Sonay, F.D., 2016. Effect of photoperiod on growth and efficiency of yolk-sac utilization in alevins of brook trout (*Salvelinus fontinalis*). Pakistan J. Zool. Pakistan Journal of Zoology 48, 533–537.

Pavlidis, M., Dimitriou, D., Dessypris, A., 1994. Testosterone and 17β-estradiol plasma fluctuations throughout spawning period in male and female rainbow trout, *Oncorhynchus mykiss* (Walbaum), kept under several photoperiod regimes. Annales Zoologici Fennici 31, 319–327.

Pickering, A., Duston, J., 1983. Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. Journal of Fish Biology 23, 163–175.

Qiu, D., Xu, S., Song, C., Chi, L., Li, X., Sun, G., Liu, B., Liu, Y., 2015. Effects of spectral composition, photoperiod and light intensity on the gonadal development of Atlantic salmon *Salmo salar* in recirculating aquaculture systems (RAS). Chinese Journal of Oceanology and Limnology 33, 45–56.

Randall, C., North, B., Futter, W., Porter, M., Bromage, N., 2001. Photoperiod effects on reproduction and growth in rainbow trout. Trout News 32, 12–15.

Reid, R., Durance, T., 1992. Textural changes of canned chum salmon related to sexual maturity. Journal of Food Science 57, 1340–1342.

Sacobie, C., Burke, H., Lall, S., Benfey, T., 2016. The effect of dietary energy level on growth and nutrient utilization by juvenile diploid and triploid brook charr, *Salvelinus fontinalis*. Aquaculture Nutrition 22, 1091–1100.

Schulz, R.W., de França, L.R., Lareyre, J.-J., LeGac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., 2010. Spermatogenesis in fish. General and Comparative Endocrinology 165, 390–411.

Skilbrei, O.T., Heino, M., 2011. Reduced daylength stimulates size-dependent precocious maturity in 0+ male Atlantic salmon parr. Aquaculture 311, 168–174.

Tam, W., Roy, R., Makaran, R., 1986. Ovarian cycle and plasma concentrations of estrogen and vitellogenin in brook trout (*Salvelinus fontinalis*, Mitchill). Canadian Journal of Zoology 64, 744–751.

Taranger, G.L., Haux, C., Stefansson, S.O., Björnsson, B.T., Walther, B.T., Hansen, T., 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17β profiles in Atlantic salmon, *Salmo salar*. Aquaculture 162, 85–98.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.-A., Dufour, S., Karlsen, Ø., Norberg, B., Angersson, E., Hansen, T., 2010. Control of puberty in farmed fish. General and Comparative Endocrinology 165, 483–515.

Taylor, J., North, B., Porter, M., Bromage, N., Migaud, H., 2006. Photoperiod can be used to enhance growth and improve feeding efficiency in farmed rainbow trout, *Oncorhynchus mykiss*. Aquaculture 256, 216–234.

Unwin, M., Rowe, D., Poortenaar, C., Boustead, N., 2005. Suppression of maturation in 2-year-old Chinook salmon (*Oncorhynchus tshawytscha*) reared under continuous photoperiod. Aquaculture 246, 239–250.

Von Saint, P., Aschoff, J., 1978. Longevity among blowflies *Phormia terraenova* R. D. kept in non-24 hour light/dark cycles. Journal of Comparative Physiology 127, 191–195.

English summary

Technology for the efficient prevention of early maturation in brook trout (*Salvelinus fontinalis* Mitchill)

Katsiaryna Lundova

Sexual maturation in salmonids has a negative impact on the general condition of fish, and as a consequence reduces the profitability of the aquaculture market as a whole. This influence can manifest itself in reduced growth rate, decreased flesh quality, increased susceptibility to diseases and assumed deterioration of the function of the immune system. Based on these aspects, delaying maturity can be considered a solution to one of the main problems in the on-growth industry. Photoperiod is an important environmental factor that elicits synchronous spawning in salmonids, and thus, as scientific evidence show, it can be effectively used to manipulate the reproduction cycle of fish.

The present thesis focuses on effect of various prolonged photoperiod regimes on sexual maturation, growth performance, flesh quality and general physical condition in brook trout (*Salvelinus fontinalis* Mitchill). In the course of the research, four different experiments were carried out using distinct strategies of the photoperiod. It is important to note that the results of this complex study revealed that the tested artificially prolonged photoperiod regimes positively influence somatic growth. In all experiments, a lower growth rate was observed in control groups, especially during spawning period. Also, it can be assumed that the use of prolonged photoperiod contributes to the delay of sexual maturation in brook trout, regardless of the sex. This statement was confirmed by the analyses of hormones level that showed a similar tendency in all parts of this study. In all of the experiments, a significantly higher number of sexually mature fish ready to spawn were found in the control groups.

The aim of the first experiment was to test the effects of an artificially prolonged photoperiod using different light sources on brook trout (Chapter 2). Two types of light sources were used, namely a light-emitting diode (LED) and a metal-halide light (HAL). In this case, the strategy of the 18-hour continued photoperiod was chosen (18L:6D). Spawning readiness, growth rate, hormone level, fins condition and colouration characteristics were studied in this experiment. Successively, an additional experiment of increasing stress for fish – transportation (a change in environmental conditions), resistance to secondary fungal disease and survival rate were analysed. More expressive spawning colouration in the control groups was confirmed by the utilize of a software (Fish Skin Colour Application software). The analysis of fin conditions in both control groups revealed the presence of longer fins. In general, minimal damage of the fins was noted during the experiment. The effect of the extended photoperiod under stress conditions manifested itself in a higher survival rate and increased resistance to fungal disease in both experimental treatments— when compared to the control groups reared under natural ambient photoperiod, regardless of sex. In this experiment, there were no significant differences between light sources in terms of intensity of influence on fish. Therefore, after an economic evaluation, light-emitting diodes can be recommended to ensure continuous photoperiod in order to delay sexual maturation and increase growth rate in brook trout.

The goal of the second experiment was to study the effect of the timing of artificially prolonged photoperiod on growth and sexual maturation of brook trout (Chapter 3). Four experimental groups were created: a control group reared under natural ambient conditions and three experimental groups exposed to an 18L:6D photoperiod regime that started at days 1, 23 and 46 of the experiment (46, 67 and 88-day post-midsummer, respectively). Light-emitting diodes were used to provide an extended photoperiod. During the study, parameters

such as spawning readiness, growth rate, hormone level, fillet yield and survival rate were analysed. Based on results obtained during this experiment, we can recommend a shorter 66-day strategy of extended photoperiod regime, starting from 19 September (88–day post–midsummer). This regime was the most cost-effective, since no difference was found among the experimental groups reared under artificial photoperiod.

Third experiment was aimed to investigate the effects of artificially prolonged non-circadian photoperiod regimes on maturity and growth rate in brook trout (Chapter 4). Three experimental treatments were exposed to prolonged light regime (24 hours of continuous exposure to light) with various non-circadian schemes that included changes of natural ambient and artificial light-dark cycles. The studied parameters, data collection and analysis were similar to those of the second experiment described above (Chapter 3). After processing all of the data and comparing the economic factors, we can recommend a non-circadian regime consisting of one artificially extended photoperiod (24L) followed by two days of natural ambient light conditions, repeating for 140 days (in total – 47 days of continuous lighting). In the experimental groups reared under this photoperiod regime, no mature fish occurred — regardless of gender. Relative to the fillet yield parameter, it should be noted that the control group of females had the lowest fillet yield throughout the experiment.

The fourth experiment was focused on the impact of extended photoperiod on post-mortem changes, and sensory, textural and nutritional characteristics of the flesh of brook trout (Chapter 5). This study was conducted after the completion of the main experiment, which was aimed at determining more effective timing regimes of artificially prolonged photoperiod regime (Chapter 3). Experimental fish were divided into four groups, according to photoperiod condition and sex. In this experiment, flesh quality was analysed via rigor index, pH changes, fillet contraction, gaping, flesh water loss, texture and colour, sensory analysis, and finally fat content and composition analyses. The results of the flesh quality testing in brook trout (Chapter 5) showed that fish exposed to artificially prolonged photoperiod had a faster progression of *rigor mortis*, significantly higher fillet contraction, less aftertaste, higher fat content and different fat composition compare to fish from the control groups.

In conclusion, the results of this study shed light on the possibility of using photo-stimulation to regulate reproductive cycles and improve growth parameters and the general characteristics of brook trout. These findings can be useful for developing an economically viable culture strategy, thus raising market profitability.

Czech summary

Technologie pro účinnou prevenci předčasného dozrávání sivena amerického (*Salvelinus fontinalis* Mitchill)

Katsiaryna Lundova

V posledních několika desetiletích bylo pohlavní dospívání lososovitých ryb identifikováno jako období, kdy dochází k negativnímu ovlivnění nejen fyziologie ryb, ale také ekonomického zisku v akvakultuře těchto ryb. Toto období se projevuje sníženou rychlostí růstu, zhoršením kvality masa, zvýšenou náchylností k nemocem a předpokládá se i celkové zhoršení funkce imunitního systému. Na základě těchto aspektů, lze oddálení pohlavní zralosti považovat za vhodné řešení hlavních problémů v podmínkách akvakultury některých druhů ryb. Fotoperioda je důležitý environmentální faktor, který způsobuje synchronizovanou reprodukci lososovitých ryb, a proto ji lze efektivně využít k manipulaci reprodukčního cyklu těchto ryb.

Na základě výše uvedených faktů je tato práce zaměřena na objasnění účinků různých režimů prodloužené fotoperiody na pohlavní zralost, růstovou schopnost, kvalitu masa a fyziologické parametry u sivena amerického (*Salvelinus fontinalis* Mitchill). V rámci této studie byly provedeny čtyři experimenty s aplikací různých režimů fotoperiody a vyhodnocení dopadů těchto zásahů. Z výsledků této komplexní práce vyplývá, že uměle prodloužená fotoperioda pozitivně ovlivňuje somatický růst, především v předvýtěrovém a výtěrovém období. V rámci všech experimentů byl pozorován nejnižší růst u kontrolních skupin. Zároveň lze konstatovat, že prodloužení fotoperidody přispívá k oddálení pohlavního zrání sivena amerického, a to bez ohledu na pohlaví. Toto tvrzení bylo podloženo analýzou hladiny pohlavních hormonů, jež vykazovala podobnou tendenci ve všech experimentech této studie. Ve všech experimentech byl v kontrolních skupinách v období výtěru nalezen výrazně vyšší počet pohlavně zralých ryb ve vyšším stupni připravenosti k reprodukci.

Cílem prvního experimentu, bylo otestovat účinky uměle prodloužené fotoperiody v chovu sivena amerického s využitím různých zdrojů světla (Kapitola 2). Byly využity dva druhy osvětlení, a sice světlo emitující diodu (LED) a halogenové světlo (HAL). V tomto případě byla zvolena strategie osmnáctihodinové fotoperiody (18L:6D). V průběhu tohoto experimentu byla sledována připravenost ryb k výtěru, rychlost růstu, přežití a intenzita zbarvení. Byla rovněž sledována odolnost ryb vůči sekundárním plísňovým onemocněním a přežití po provedení experimentu zaměřeného na podmínky zvýšeného stresu při přepravě ryb (změny podmínek prostředí). V rámci experimentu s různými světelnými zdroji se pomocí software (Fish Skin Colour Evaluation Application software) bylo potvrzeno výraznější zbarvení (svatební šat) v kontrolních skupinách. Za zvýšeného stresu se prodloužená fotoperioda v obou experimentálních skupinách projevila ve zvýšeném přežití a odolnosti vůči plísňovým chorobám ve srovnání s jedinci chovanými v podmínkách přirozené fotoperiody, a to bez ohledu na pohlaví (Kapitola 2). Vzhledem k absenci výrazných rozdílů v efektivitě oddálení puberty při testování vlivu různých zdrojů světla, lze po ekonomických výpočtech doporučit LED světla pro zajištění podmínek prodloužené fotoperiody s cílem oddálení pohlavní zralosti a zvýšení rychlosti růstu sivena amerického ve finálních fázích odchovu.

Cílem druhého experimentu bylo testování vlivu načasování uměle prodloužené fotoperiody na růst, výtěžnost filet, dynamiku pohlavních hormonů, připravenost k výtěru a pohlavní zralost sivena amerického (Kapitola 3). Byly založeny čtyři experimentální skupiny: kontrolní skupina chovaná v přirozených světelných podmínkách a tři skupiny vystavené fotoperiodě v režimu 18L:6D počínající dnem 1., 23. a 46. dnem (46., 67. a 88. den letního slunovratu) pokusného období. Pro zajištění prodloužené fotoperiody byla použita LED světla. Na základě

analyzovaných údajů získaných během experimentu lze doporučit nejkratší šestašedesátidenní strategii prodlouženého režimu fotoperiody s počátkem od 19. září (88. den letního slunovratu). Tento režim se ukázal jako ekonomicky nejefektivnější a přináší srovnatelné efekty na proces dozrávání, vývoj gonád a připravenost k výtěru.

Třetí experiment byl zaměřen na vliv non-cirkadiálních režimů fotoperiody na pohlavní zralost a růstové parametry sivena amerického (Kapitola 4). Tři testované skupiny byly vystaveny prodlouženému světelnému režimu (24 hodin světla – 24L) s různými non-cirkadiálními režimy změny přirozeného a umělého cyklu světlo/tma. Testované parametry, sběr a následná analýza dat probíhaly analogicky s druhým experimentem (Kapitola 3). Po zpracování všech dat a zohlednění ekonomických nákladů lze doporučit režim s non-cirkadiální fotoperiodou sestávající se z jednoho uměle prodlouženého dne (24L), následovaného dvěma dny přirozených světelných podmínek s opakováním po dobu 140 dnů (celkem – 47 dní nepřetržitého osvětlení). Analýzy výtěžnosti filet prokázaly, že kontrolní skupina jikernaček dosahovala během experimentu nejnižší výtěžnosti.

Čtvrtý experiment byl zaměřen na postmortální změny, senzorické, texturní a nutriční vlastnosti masa sivena amerického (Kapitola 5) po předchozím odchovu v podmínkách prodloužené fotoperiody. Tato studie byla provedena po dokončení hlavního experimentu, jehož cílem bylo stanovit účinnější strategie načasování uměle prodloužené fotoperiody (Kapitola 3). Experimentální ryby byly rozděleny do čtyř skupin podle režimů fotoperiody a pohlaví. V rámci této studie byla kvalita masa analyzována prostřednictvím indexu *rigor mortis*, změn pH, kontrakce filet, mezerovitosti (gaping), úbytku vody ve svalovině, textury a barvy, senzorických vlastností, obsahu tuku a celkového nutričního složení. Výsledky testování kvality masa sivena amerického (Kapitola 5) prokazují, že experimentální skupiny vystavené uměle prodloužené fotoperiodě vykazovaly rychlejší postup *rigor mortis*, signifikantně vyšší kontrakci filet, lepší senzorické vlastnosti, vyšší obsah tuku a rozdíly složení tuku v porovnání s rybami z kontrolních skupin.

Závěrem lze říci, že výsledky této studie explikují možnost využití fotostimulace k regulaci reprodukčních cyklů, zlepšení růstových parametrů, výtěžnosti a kvality masa u sivena amerického, především ve finálních fázích odchovu. Tato zjištění mohou být užitečná pro další rozvoj intenzivní akvakultury a lepší uplatnění sivena amerického na trhu.

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List of publications

Peer-reviewed journals with IF

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Prokešová, M., Gebauer, T., Matoušek, J., **Lundova, K**., Čejka, J., Zusková, E., Stejskal, V, 2020. Effect of temperature and oxygen regime on growth and physiology of juvenile Salvelinus fontinal*is* × *Salvelinus alpinus* hybrids. Aquaculture 522: 735–119 (IF 2019 = 3.224)

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Linhartova, Z., Lunda, R., Masilko, J., Dvorak, P., **Lundova, K**., Stejskal, V., Matousek, J., Mraz, J., 2018. Impact of photostimulation for delayed maturity on flesh quality of brook trout (*Salvelinus fontinalis*) stored under refrigerated conditions. Aquaculture Research 49: 3817–3829. (IF 2017 = 1.475)

Lundova, K., Kouril, J., Sampels, S., Matousek, J., Stejskal, V., 2018. Growth, survival rate, and fatty acids composition of starlet (*Acipenser ruthenus*) larvae fed fatty acid enriched *Artemia nauplii*. Aquaculture Research 49: 3309–3318. (IF 2017 = 1.475)

Sebesta, R., Stejskal, V., Matousek, J., **Lundova, K**., 2018. The effect of light intensity and tank colour on survival and growth of Peled *Coregonus peled* Gmelin 1788 larvae. Turkish Journal of Fisheries and Aquatic Sciences 19: 541–549. (IF 2017 = 0.482)

Matousek, J., Prokesova, M., **Novikava, K**., Sebesta, R., Zuskova, E., Stejskal, V., 2017. The effect of water oxygen saturation on growth and haematological profile of juvenile peled *Coregonus peled* (Gmelin). Aquaculture Research 48: 5411–5417. (IF 2016 = 1.461)

Peer-reviewed journals without IF

Svinger, V., Stejskal, V., Prokesova, M., Matousek, J., Sebesta, R., **Novikava, K**., Kouril, J., 2015. Verzogerung der Geschlechtsreifung bei zweisommerigen Bachsaiblingen durch Einsatz zweier verschiedenen Beleuchtungssysteme. Fischer & Teichwirt 5: 163–166.

Applied methodologies, patents, pilot plants, verified technologies

Stejskal, V., Matoušek, J., Šebesta, R., **Novikava, K**., Prokešová, M., Mareš, J., 2015. Postupy pro efektivní odchov larev síha peledě (*Coregonus peled* Gmelin) v intenzivních podmínkách. Mendelova univerzita v Brně. Metodika R11/2015. 22 pp.

Abstracts and conference proceedings

Lundova, K., Kouřil, J., Matoušek, J., Prokešová, M., Stejskal, V., 2018. Vliv různých zdrojů osvětlení, fotoperiodických režimů a načasování prodloužené fotoperiody na růst a pohlavní zralost sivena amerického (*Salvelinus fontinalis*, Mitchill). In: Sborník příspěvků z workshopu „Zvýšení a zefektivnění produkce lososovitých ryb v ČR s využitím jejich genetické identifikace, 14 November 2018, Mendelova univerzita v Brně, pp. 63–73.

Prokešová, M., Kouřil, J., **Novikava, K**., Čejka, J., Zusková, E., Stejskal, V., 2018. Vliv hyperoxických režimů a teploty vody na fyziologii a produkci juvenilů sivena alsaského. In: Sborník příspěvků z workshopu „Zvýšení a zefektivnění produkce lososovitých ryb v ČR s využitím jejich genetické identifikace, 14. November 2018, Mendelova univerzita v Brně, pp. 55–62.

Dvorak, P., Lunda, R., Linhartova, Z., **Novikava, K**., Mraz, J., Dvorakova-Liskova, Z., 2017. The use of biofloc biomass as a component of feed for juvenile grass carp (*Ctenopharyngodon idella*) in RAS. In: EAS (Eds), Aquaculture Europe 2017. 17–20 October 2017, Dubrovnik, Croatia, pp. 312–313.

Linhartová, Z., Lunda, R., Másílko, J., Dvořák, P., **Novikava, K**., Stejskal, V., Matoušek, J., Mráz, J., 2017. Impact of photostimulation for delayed maturity on flesh quality of brook trout (*Salvelinus fontinalis*) stored under refrigerated conditions. In: EAS (Eds), Aquaculture Europe 2017. 17–20 October 2017, Dubrovnik, Croatia, pp. 672–673.

Lundova, K., Matousek, J., Prokesova, M., Sebesta, R., Vanina, T., Stejskal, V., 2017. The effect of timing of photoperiod prolongation on postponement of puberty in brook trout (*Salvelinus fontinalis*). In: EAS (Eds), Aquaculture Europe 2017, 17–20 October, Dubrovnik, Croatia, pp. 686–687. (Poster presentation)

Lundova, K., Stejskal, V., Sebesta, R., Matousek, J., 2017. The effect of non-circadian regimes on growth and puberty of brook trout (*Salvelinus fontinalis* Mitchill). VI International Young Researchers' Conference of NACEE, 28 November – 1 December 2017, Gorki (Republic of Belarus).

Stejskal, V., **Lundova, K**., Sebesta, R., Matousek, J., Vanina, T., Roje, S., 2017. Different response of pure Arctic char *Salvelinus alpinus* and hybrid (*Salvelinus alpinus* vs. *Salvelinus fontinalis* Mitchill) to various hyperoxic regimes. ICAS 2017: 19th International Conference on Aquaculture and Fisheries, 18–19 December 2017, Bangkok, Thailand, urn :dai:10.1999/1307-6892/82768.

Stejskal, V., Matousek, J., Prokesova, M., **Novikava, K**., Vanina, T., Sebesta, R., Gasco, L. 2017. The effect of different insect meal (*Hermetia ilucens*) inclusion on growth performance of Eurasian perch (*Perca fluviatilis* L.). In: EAS (Eds), Aquaculture Europe 2017. Dubrovnik, Croatia, 17–20 October 2017, pp. 1100–1101.

Matousek, J., Prokesova, M., **Novikava, K**., Sebesta, R., Zuskova, E., Kouril, J., Stejskal, V., 2016. Vliv teploty vody a koncentrace kyslíku na výsledky chovu síha peledě v intenzivní akvakultuře. In: Mareš, J., Lang, Š., Marešová, M.G. (Eds), Zkušenosti s chovem ryb, optimalizací prostředí a veterinární péčí v recirkulačních systémech. Brno 15. November 2016, pp. 85–94.

Novikava, K., Stejskal, V., Kouril, J., Matousek, J., 2016. Enrichment of *Artemia* nauplii with different diets: effect of growth rate, survival and fatty acids composition of the sterlet larvae (*Acipenser ruthenus*). In: EAS (Eds), Aquaculture Europe 2016. 20–23 September 2016, Edinburgh, Scotland (Great Britain), pp. 698–699.

Prokesova M., Stejskal V., Matousek J., Vanina, T., Sebesta, R., **Novikava, K**., Kouril J. 2016. Effect of different light conditions on early development of African sharptooth catfish *Clarias gariepinus*. In: Aquaculture Europe 2016, 20–23 September 2016, Edinburgh, Scotland (Great Britain), pp. 816–817.

Šebesta, R., Stejskal, V., Kouřil, J., Prokešová, M., Matoušek, J., **Novikava, K**., Vanina, T., 2016. Combined effect of water temperature and tank shape on performance of Peled (*Coregonus peled* Gmelin, 1788) larvae. In: Proc. Workshop on the Biology and Management of Coregonid fishes. Tyumeń (Russia), 2 p.

Sebesta, R., Stejskal, V., Matousek, J., Prokesova, M., **Novikava, K.,** 2016. The combined effect of light intensity and tank wall colour on growth and survival of peled (*Coregonus peled* Gmelin, 1788) larvae. In: Aquaculture Europe 2016, 20–23 September 2016, Edinburgh, Scotland (Great Britain), pp. 909–910.

Šebesta, R., Stejskal, V., Prokešová, M., **Novikava, K**., Vanina, T., 2016. Vliv teploty vody a tvaru nádrže na růst, přežití, a frekvenci výskytu deformit čelistí u larev síha peledě (*Coregonus peled* Gmelin 1788). In: Mareš, J., Lang, Š., Marešová, M.G. (Eds), Zkušenosti s chovem ryb, optimalizací prostředí a veterinární péčí v recirkulačních systémech. Brno 15. November 2016, pp. 76–84.

Stejskal, V., Matousek, J., Prokesova, M., Sebesta, R., **Novikava, K**., Podhorec, P., Zajic, T., Kouril, J., 2015. Fatty acids profiles and proximate composition of peled (*Coregonus peled* Gmelin) fillets originated from two culture systems. In: EAS (Eds), Aquaculture Europe 2015. Rotterdam (Netherland), 20–23 October 2015.

Chepurkina, M., **Novikava, K**., Kouřil, J., 2014. Wykorzystanie metody bioenkapsulaciji naupoliusów solowca (*Artemia*) w żywieniu larw ryb w waruńkach kontrolowanego środowiska (Studium przegladowe). In: Proc. Abstr. konf. Inżynieria akvakultury, UWM, Olsztyn (Poland) 2–4 December 2014, 1 p.

Training and supervision plan during study

Name	M.Sc. Katsiaryna Lundova
Research department	2014–2020 – Laboratory of Controlled Reproduction and Intensive Fish Culture of FFPW USB
Supervisor	Vlastimil Stejskal, Ph.D.
Period	29th October 2014 until 16th September 2020

Ph.D. courses	**Year**
Intensive fish breeding	2015
Fish nutrition	2015
Biostatistics	2015
Ichthyology and fish taxonomy	2016
Basic of scientific communication	2016
English	2019

Scientific seminars	**Year**
Seminar days of FFPW	2015 2016 2017 2017

International conferences	**Year**
Novikava, K., Stejskal, V., Kouril, J., Matousek, J. Enrichment of *Artemia* nauplii with different diets: effect of growth rate, survival and fatty acids composition of the sterlet larvae (*Acipenser ruthenus*). In: EAS (Eds), Aquaculture Europe 2016. 20–23 September 2016, Edinburgh, Scotland (Great Britain), pp. 698–699.	2016
Lundova, K., Matousek, J., Prokesova, M., Sebesta, R., Vanina, T., Stejskal, V. The effect of timing of photoperiod prolongation on postponement of puberty in brook trout (*Salvelinus fontinalis*). Poster presentation. In: EAS (Eds), Aquaculture Europe 2017. Dubrovnik, Croatia, 17–20 October 2017, pp. 686–687.	2017
Lundova, K., Stejskal, V., Sebesta, R., Matousek, J. The effect of non-circadian regimes on growth and puberty of brook trout (*Salvelinus fontinalis* Mitchill). VI International Young Researchers' Conference of NACEE, 28 November – 1 December 2017, Gorki (Republic of Belarus).	2017

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Prof. Dr. Gilbert Van Stappen, Laboratory of Aquaculture and *Artemia* Reference Center, Ghent University, Gent, Belgium (2 weeks, studying the new methods of enrichment of *Artemia* nauplii)	2016
Prof. Ryszard Kolman, Department of Ichthyology, The Stanislav Sakowicz Inland Fisheries Institute, Olsztyn, Poland (1 month, studying the new methods of enrichment of *Artemia* nauplii)	2016
Gerrit Timmerhaus, Ph.D., Nofima, Ås, Norway (1 month, studying the methods of gut and skin flora analysing)	2017

Pedagogical activities	**Year**
Lecturing of students of "ERASMUS" programme, Culture of Salmonids (UA/CSA), FFPW USB, winter semester	2015
Leading of student project entitled Experimental larval rearing of Siberian sturgeon (*Acipenser baerii*) and tench (*Tinca tinca*) using enriched live food (*Artemia s.*)	2015
Announcing the project entitled Enrichment of *Artemia*: how different nutritional diets affect the quality and growth rate within International fishery summer school	2016
Lecturing of field excursion with student from "Yspertal" workshop programme (Austria)	2016–2018
Consultancy of three B.Sc. theses	2016–2018
Supervision of one B.Sc. thesis	2017–2018

Curriculum vitae

PERSONAL INFORMATION

Name: Katsiaryna
Surname: Lundova (Novikava)
Title: M.Sc.
Born: 17th November 1990, Kirovograd, UKR
Nationality: Belarusian
Languages: Belarusian, Russian, English (B2), Czech (B1)
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EDUCATION

2014 – present Ph.D. student, Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses (CENAKVA), Czech Republic

2013–2014 M.Sc., Belarusian State Agricultural Academy, Department of Ichthyology and Industrial fish breeding, The Republic of Belarus

2008–2013 B.Sc., Fishery, Belarusian State Agricultural Academy, Department of Ichthyology and Industrial fish breeding, The Republic of Belarus

COMPLETED COURSES

16/02–28/02 2016 Training course in the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Gent, Belgium

09/09 2016 Course for sensory analysis evaluator, Mendel University, Brno, Czech Republic

RESEARCH STAY AND COLLABORATIONS

Foreign stays during Ph.D. study at FFPW

16/02–28/02 2016 Prof. Dr. Gilbert Van Stappen, Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Gent, Belgium (2 weeks, studying the new methods of enrichment of Artemia nauplii)

25/10–25/11 2016 Prof. Ryszard Kolman, Department of Ichthyology, The Stanislav Sakowicz Inland Fisheries Institute, Olsztyn, Poland (1 month, studying the new methods of enrichment of Artemia nauplii)

04/09–04/10 2017 Gerrit Timmerhaus, Ph.D., Nofima, Ås, Norway (1 month, studying the methods of gut and skin flora analysing)