

# Photo-thermal manipulation for the reproductive management of pikeperch *Sander lucioperca*

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**Abstract** The aim of the present study was to investigate the effect of photoperiod regime combined with thermal treatment (PTT) on maturation of three-year-old pikeperch (*Sander lucioperca*). To induce the onset of maturation, all treatment groups underwent a wintering phase of 3 months at 12 °C and 12-h light-to-12-h dark. Controls were maintained at 23 °C and 12-h light-to-12-h dark throughout the trial. After the wintering phase, the PTT groups experienced regimes with 8-, 10-, 12-, and 14-h light for 4 months at 14 °C referred to as photo-thermal treatment (PTT). The four PTT groups as well as the control were studied in triplicates, with 12 fish per tank. We assessed gonad development and maturation by histological analysis, sex steroid plasma concentrations (17 $\beta$ -estradiol, testosterone, and 11-ketotestosterone) and, at the end of the experiment, mRNA expression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the pituitary. As expected, both male and female pikeperch initiated reproductive maturation during the wintering phase, which was confirmed through histological analysis and sex steroid measurements. During wintering, the onset of maturation in females was confirmed by the increasing diameter of ovarian follicles (217  $\pm$  65 to 800  $\pm$  97  $\mu$ m), the developmental stages (50 % mid and 21 % late vitellogenic females), concomitant with elevated 17 $\beta$ -estradiol (E2) plasma concentrations reaching 2600  $\pm$  1500 pg/mL, compared to 380  $\pm$  230 pg/mL at the onset of the experiment. Similarly in males, maturation was indicated by peak

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concentrations of the androgens observed within the first 2 months of wintering (testosterone  $21 \pm 12$  ng/mL first month, 11-ketotestosterone  $5.7 \pm 3$  ng/mL second month). Subsequently, the photoperiod treatments after the wintering phase influenced the progression of reproductive maturation. During the PTT, follicle diameter increased irrespective of the light regime from 800 to more than 1100  $\mu\text{m}$ , but the progression of the vitellogenesis was differentially modulated by the photoperiod. Already after 1 month of the PTT, 92 and 86 % of females reared at 12- and 14-h light per day were in a late vitellogenic stage. After 2 months, females with mainly atretic follicles were observed under long light conditions (14 h of light), indicating overripeness and spawning. In contrast, 82 and 72 % of the females reared at 8- and 10-h light per day were still in the final stage of vitellogenesis at the end of the experiment and thus ready to undergo final maturation. Concomitant to the histological outcomes, highest E2 concentrations were observed under long-day conditions (12, 14 h) in females with peaks of  $4200 \pm 3300$  (12 h of light) and  $6800 \pm 4200$  pg/mL (14 h of light). In male pikeperch, ongoing spermatogenesis was indicated by rising androgen levels especially under long-day conditions (14 h of light) reaching peak levels of  $27 \pm 21$  ng/mL testosterone and  $39 \pm 91$  ng/mL of 11-ketotestosterone at the end of the experiment. In all PTT, the mRNA expression of FSH and LH was significantly elevated compared to the control, confirming the activation of the hypothalamus–pituitary–gonad axis. Here, no effect between the different light regimes was detected, neither in males nor females. Thus, photoperiod revealed a slight influence on male and female pikeperch maturation when combined with an effective thermal treatment and therefore can be explored as a cheap and easy-to-handle fine-tuning tool for artificial pikeperch reproduction.

**Keywords** Out-of-season reproduction · Photoperiod · Vitellogenesis · Spermatogenesis · Sex steroids

### Abbreviations

11-KT	11-Ketotestosterone
17,20-P	17,20 $\beta$ -Progesterone
E2	17 $\beta$ -Estradiol
FSH $\beta$	Follicle-stimulating hormone $\beta$ -subunit
HPG axis	Hypothalamus–pituitary–gonad axis
LV	Late vitellogenic follicles
LH $\beta$	Luteinizing hormone $\beta$ -subunit
n. c.	Natural conditions
n. s.	Not specified
PTT	Photo-thermal treatment
RAS	Recirculating aquaculture system
T	Testosterone
us.	Unspecified

### Introduction

Currently, aquaculture is the fastest growing sector in agriculture, but, to date, Europe (with few exceptions such as Norwegian salmon aquaculture) does not participate in this development. In the last decades, increasing attention has been given to the evaluation and introduction of new species for European aquaculture production. Here, candidate species

are highly valuable to the market, but often facing problems with regard to the aquaculture technology, for example Burbot (*Lota lota*) (Barron et al. 2012; Wocher et al. 2012; Palinska-Zarska et al. 2014, 2015), Eurasian perch (*Perca fluviatilis*) (Abdulfatah et al. 2013; Pimakhin et al. 2015), and also pikeperch (*Sander lucioperca*) (Zakes 2007; Mueller-Belecke and Zienert 2008; Szczepkowski et al. 2011; Zarski et al. 2012, 2013).

In Europe, aquaculture production is mainly based on open systems such as ponds, net cages, or raceways, but due to limited resources (water, sites) required for open systems (ponds, net cages), the expansion of production in closed recirculation aquaculture systems (RAS) is envisioned for the future. These land-based systems allow a specific control over the rearing environment, but are challenged by the economic feasibility. Here, control of the production cycle is a key issue which mainly relies on the control and management of reproduction allowing the stocking independently of the season. Consequently, it is essential to identify the species-specific cues triggering reproduction. In temperate species, the reproductive cycle is usually synchronized by seasonal triggers, predominantly light and temperature (Taranger et al. 2010; Wang et al. 2010). The role of these external factors may be species-specific, and reproductive management may involve different triggers at different periods of the reproductive cycle or even a combination of different environmental cues.

In salmonids for instance, photoperiodic change is considered to be the main factor controlling the synchronization of the reproductive cycle (Bromage et al. 2001; Migaud et al. 2010). In temperate species, photoperiod has been regarded as the principal environmental determinant of reproduction, and temperature has often been considered as a secondary cue (Bromage et al. 2001; Pankhurst and Porter 2003). Yet, most of the information on the role of temperature is derived from cyprinids where rising temperatures in spring initiate gonad maturation (Stacey 1984; Pankhurst and Porter 2003).

In freshwater percids, both temperature and photoperiod factors play a key role in the regulation of the reproductive cycle (Taranger et al. 2010; Wang et al. 2010). For example, gonad maturation in Eurasian perch (*P. fluviatilis*) is inhibited by a continuous photophase, and optimal maturation is only achieved under a natural photoperiod (Migaud et al. 2006). At a constant photoperiod (16-h light-to-8-h dark), maturation was heterogeneous, suggesting that a decreasing photoperiod is the environmental trigger for the initiation of gonad maturation. These experimental fish were kept at ambient temperatures (15–23 °C), thereby suggesting that a distinct wintering as previously described by Migaud et al. (2002, 2004a, b) was not required for the initiation of puberty (in females the transition from previtellogenesis to vitellogenesis as discussed in Okuzawa 2002; Wuertz et al. 2007). Thus, in Eurasian perch (*P. fluviatilis*), natural photoperiod seems to play a key role in out-of-season reproduction, and decreasing day lengths are sufficient to induce puberty.

Nevertheless, our previous investigations found temperature to be the crucial factor either to induce puberty or to arrest gonad development in the pikeperch (Hermelink et al. 2011, 2013). Subsequently, temperature also synchronizes gonadal development in pikeperch of both sexes (Hermelink et al. 2013). As a result of these studies, we presented an optimized protocol: a wintering period at 12 °C for 3 months, followed by a slight temperature increase to 14 °C for a further 4 months to optimally support the progression of gametogenesis. Divergence of temperatures from this optimized protocol (e.g., lower temperature for wintering or higher temperatures for maturation) resulted in suboptimal maturation and, at 23 °C, resulted in a complete stagnation of gametogenesis. Both experiments were carried out under a fixed photoperiod of 12-h light and 12-h dark. However, despite the technical ease of installation, low cost, and its potential for

reproductive management, the influence of the photoperiod has yet to be investigated in pikeperch.

Therefore, in the present study, the effect of the photoperiod was evaluated experimentally. Maturation was induced and supported by the optimal thermal protocol (Hermelink et al. 2011, 2013) in four experimental groups reared at day lengths of 8-h light and 16-h dark (08:16), 10-h light and 14-h dark (10:14), 12-h light and 12-h dark (12:12), and 14-h light and 10-h dark (14:10) after wintering at 12:12 in one recirculating aquaculture system (RAS). We characterized the maturational process by assessing gonadotropin gene expression in the pituitary, sex steroids in the plasma and developmental stages of the ovary.

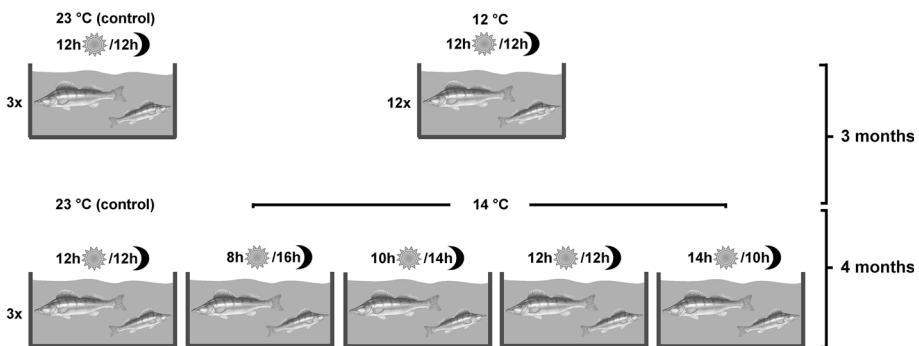
## Materials and methods

### Animal rearing and experimental setup

Pikeperch (aged 10–11 months) were obtained from a commercial producer (Fischerei Mueritz-Plau GmbH, Waren, Germany). Before onset of the experiment, these fish were kept at the facilities of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries for over 2 years under controlled conditions (daily artificial illumination of 12:12 and a constant water temperature of 23 °C). Prior to the experiment, 12 randomly chosen pikeperch were stocked to each of fifteen 500-L tanks of the same RAS, thus providing four photo-thermal treatment (PTT) groups and one control group (23 °C, 12:12), assessed in triplicate (Fig. 1).

Females had an average size of  $52 \pm 6$  cm and a mean weight of  $1300 \pm 440$  g, and males were slightly bigger with an average size of  $54 \pm 7$  cm and a mean weight of  $1510 \pm 590$  g (sex of fish was assessed by histological analysis). To avoid the influence of artificial light emission from surrounding rearing units, all tanks were covered with light-tight material and illuminated artificially (Aqua medic aqualine T5 Reef White 10.000 K) for the duration of the experiment.

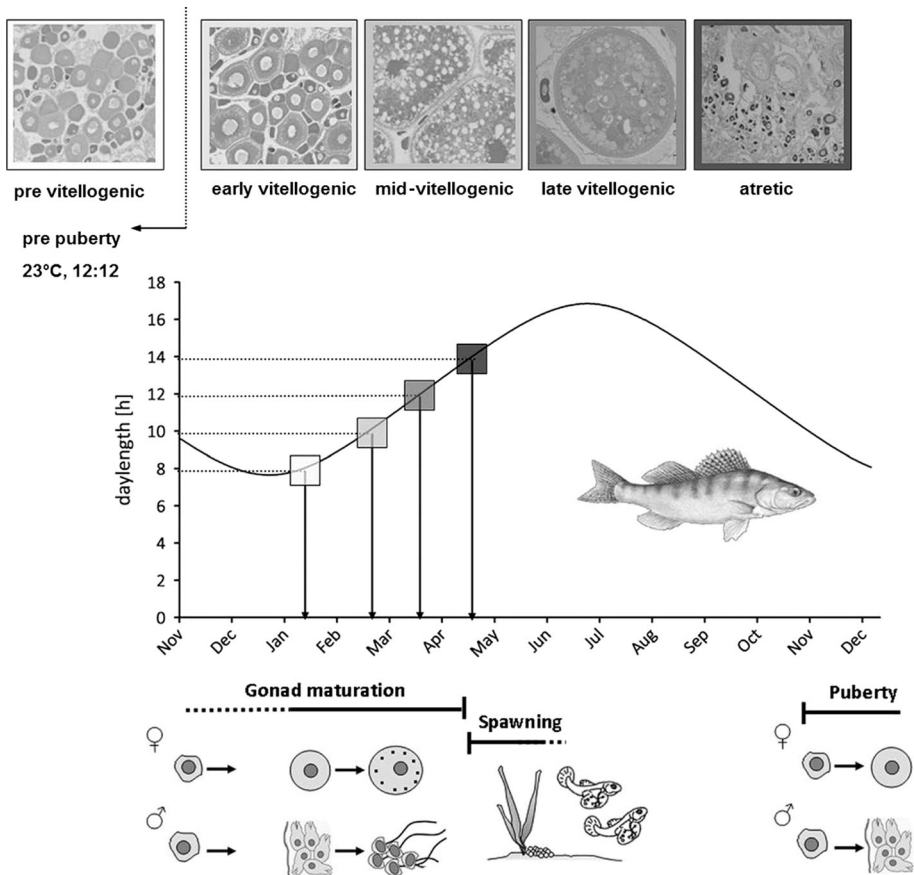
On commencing the experiment (day zero—2011.12.01), an optimized thermal protocol was applied to all four experimental groups (except the control group)—starting in



**Fig. 1** Experimental setup: The effect of four different photoperiods (photo-thermal treatment at 14 °C and 08:16, 10:14, 12:12, or 14:10) on the progression of gonad maturation was studied in three-year-old pikeperch after gonad maturation was induced by wintering at 12 °C for 3 months. A fifth group was kept as a control at 23 °C and a photoperiod of 12:12

December with 14 d of cooling down from 23 to 12 °C and a subsequent wintering at 12 °C for 3 months—according to Hermelink et al. (2011) (Fig. 1).

Following completion of the wintering period in March, the temperature was adjusted to 14 °C which has been identified as optimal temperature to support the maturation of pikeperch according to Hermelink et al. (2013). The rise in temperature from 12 to 14 °C was established within 1 week and maintained for an additional 4 months. During these 4 months, the four different experimental groups were exposed to either 8, 10, 12, or 14 h of light per day to evaluate the effect of the photoperiod on gonad maturation (Fig. 1). For all respective groups (except the control fish and the fish of the 12:12 group), the illumination time was adjusted on a daily basis after wintering to finally establish the designated day length within the first month. The experimental photoperiods were chosen according to natural conditions observed between December and May, when the onset of maturation (in females transition from previtellogenesis to vitellogenesis), maturation (in females vitellogenesis), and final maturation takes place in wild stocks (Fig. 2). Fish were fed on a commercial trout diet (DAN-EX 1750, Dana Feed/Biomar: 16 % carbohydrates,



**Fig. 2** Photoperiod during the natural reproductive cycle of the pikeperch assigned to the transition from previtellogenesis (puberty), vitellogenesis (maturation), and final maturation and ultimately spawning. The dotted lines identify the experimental photoperiod assessed here (8-, 10-, 12-, and 14-h day length), assigned to the histological stage

17 % fat, and 50 % protein) at a daily ratio of 0.5 % of whole body mass, adjusted after each sampling point (monthly, see below).

### Sampling procedure

All fish were sampled at day zero and then again at the end of the experiment in early July (2012.07.04). During the 3-month wintering phase,  $5 \pm 1$  fish from each tank of the PTT groups and  $8 \pm 1$  fish from the control were sampled after each month. During the 4-month PTT,  $8 \pm 1$  fish from each of the treatment and control tanks were sampled after each month (the number of female and male pikeperch sampled at the eight different sampling points is given in Tables 1, 2). If a fish died during anesthesia or sampling, it was excluded from the analysis.

Prior to sampling, fish were anesthetized in a MS222-bath (50 mg/L) for two to 3 min until loss of eye reflex and tail fin movement. Total length was taken to the nearest 0.5 cm from the tip of the snout to the end of the longest lobe of the tail fin. Wet weight was measured to the nearest g.

Blood was taken from the caudal vein using heparinized 2-mL syringes and immediately transferred to 2-mL Safe-Lock Tubes (Eppendorf) and centrifuged for 5 min (8000g). The cell-free plasma was separated in 400  $\mu$ L aliquots, shock-frozen in liquid nitrogen, and stored at  $-80$  °C for further analysis. Follicle samples were taken by introduction of a flexible catheter ( $\varnothing$  2.2 mm) into the genital porous according to Steffens et al. (1996), Mueller-Belecke and Zienert (2008) and Zakes and Demska-Zakes (2009). Average follicle size was measured for 20 randomly chosen follicles of the largest cohort per female using an Olympus RX50 microscope equipped with an Olympus XC50 digital camera. Subsequently, follicles were transferred to Bouin's solution for histological preparation. After the examination, fish were allowed to completely recover in aerated tanks before they were returned to their respective treatment tank.

After 7 months, at the end of the experiment, all fish were killed for the final sampling with an overdose of MS222-bath (250 mg/L). The pituitary was dissected, shock-frozen in liquid nitrogen, and stored at  $-80$  °C for gene expression analysis of the gonadotropins FSH and LH.

### Gonad histology

Sex was determined upon biopsy. After fixation in Bouin's solution for 12 h, samples were dehydrated in a graded series of ethanol and embedded in paraffin (Paraplast, Roth,

**Table 1** Number of female pikeperch sampled according to treatment and sampling point

Treatment	Sample point (months)							
	Wintering				Photo-thermal treatment			
	0	I	II	III	IV	V	VI	VII
23 °C	102	9	9	10	11	9	10	13
12:12								
08:16					17	13	16	17
10:14					15	14	15	20
12:12		50	51	58	13	16	15	18
14:10					15	15	18	22

**Table 2** Number of male pike-perch sampled according to treatment and sampling point

Treatment	Sample point (months)							
	Wintering				Photo-thermal treatment			
	0	I	II	III	IV	V	VI	VII
23 °C 12:12	25	12	15	15	15	15	14	19
08:16					8	10	8	11
10:14					9	10	9	13
12:12		10	10	10	11	8	9	17
14:10					9	9	6	12

Germany). Subsequently, at least 40 slides were cut at approximately 5 µm from each sample and stained with hematoxylin–eosin. The staging of female gonads was performed as described in Hermelink et al. (2011, 2013) on the basis of Selman et al. (1993) and Lubzens et al. (2010). Individuals were classified as pre, early, mid, late vitellogenic or atretic if more than 50 % of the follicles fulfilled the characteristics of the respective stage according to Table 3. For each sample, 20 randomly chosen follicles of four different histological slides were examined. Due to the applied noninvasive sampling technique, the amount of tissue obtained from male pikeperch was only sufficient for the determination of the sex but not for a reasonable staging assessing testis stages (e.g., spermatocysts, spermatids, sperm) quantitatively.

**RNA extraction and reverse transcription**

Extraction of total RNA from pituitaries was performed using the RNeasy extraction kit (Qiagen) including on-column DNase digestion according to Trubiroha et al. (2009). The total RNA concentration was evaluated by UV absorption spectrometry with a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific). The integrity of the RNA was assessed as the RNA integrity number (RIN) for a randomly chosen set of RNA samples on RNA 6000 Nano LabChips with an Agilent 2100 bioanalyzer (Agilent Technologies). The measured RIN values were always higher than 8.5 confirming RNA quality for subsequent

**Table 3** Description of ovarian stages used for classification

Previtellogenesis	Early vitellogenesis	Mid-vitellogenesis	Late vitellogenesis	Atretic
Diameter max. 250 µm Cytoplasm is either free of cortical alveoli or nascent cortical alveoli, distributed within the oocytes, close to the cortical cortex	Diameter max. 400 µm Accumulation of lipid droplets in the cytoplasm First vitellogenin droplets	Diameter max. 700 µm Oocytes filled with numerous small lipid and yolk vesicles Yolk vesicle accumulate centripetally	Diameter > 700 µm Oocytes filled with few large lipid and yolk vesicle Yolk accumulates centripetally All oocyte layers (theca cells, the basement lamina and the granulosa cells) developed incl. jelly coat	Loss of cell integrity Globular shape Layering disintegrates Cytoplasm becomes matrix-like

RT-qPCR analysis (Fleige and Pfaffl 2006). Reverse transcription was carried out in a two-step reaction using AMV-RT (Finnzymes). First, 1  $\mu\text{g}$  of total RNA was incubated with 7.5 pmol oligo-dT primer (5'-CCTGAATTCTAGAGCTCA(T)<sub>17</sub>-3') at 70 °C for 3-min primer annealing (20  $\mu\text{L}$  final volume), followed by cooling on ice to stabilize hybrid molecules for 10 min. Subsequently, reverse transcription was carried out after the addition of 15 nmol dNTP (each), 10 U AMV-RT, and 3  $\mu\text{L}$  10 $\times$  reaction buffer (250 mM Tris HCl, 500 mM KCl, 20 mmol/L DTT, 50 mM MgCl<sub>2</sub>, pH 8.3) with a final volume of 30  $\mu\text{L}$  at 36 °C for 60 min and then at 94 °C for 3 min. A negative RT control, where the AMV-RT had been replaced by RNase-free water, was included to rule out genomic DNA contamination of the samples.

### Gene expression analysis by real-time quantitative PCR (qPCR)

Expression of FSH $\beta$  and LH $\beta$ -subunits using ribosomal protein L8 (rpL8) as a housekeeping gene was carried out as described in Hermelink et al. (2011). Relative quantification assays were performed with a Mx3005 qPCR Cyclor (Stratagene) using hot start polymerase (Platinum, Invitrogen) and SYBR Green as fluorescent dye in a 20- $\mu\text{L}$  reaction volume (2  $\mu\text{L}$  diluted cDNA, 7.5 pmol of each primer, 1 $\times$  Taq buffer, 2 mM MgCl<sub>2</sub>, 1.7 nmol of each dNTP, 0.1  $\mu\text{L}$  of 200-fold diluted SYBR Green I solution, ROX reference dye, 1 U polymerase). Cycling protocol comprised an initial denaturation and activation of the hot start polymerase at 95 °C for 7 min, followed by 40 amplification cycles of 95 °C denaturation for 20 s, 62 °C primer annealing for 25 s, and 72 °C extension for 25 s. PCRs were always run in duplicate. The expression was determined by the comparative C<sub>T</sub> method ( $\Delta\Delta C_T$ ) according to Pfaffl (2001) with regard to a calibrator sample (pooled pituitary cDNA) compensating for run-to-run variations and corrected for PCR efficiencies of the target genes. Upon validation of even expression in the samples, rpL8 was used as a housekeeping gene. Specificity of the assays was confirmed by direct sequencing and controlled by melting curve analysis.

### Steroid analysis

Steroid determination was carried out by hormone-specific ELISA (Cayman) as previously described (Hermelink et al. 2011, 2013). In summary, 100  $\mu\text{L}$  of plasma was extracted twice with 1 mL of diethyl ether (Roth) in a glass vial for 1 min. Afterward, the samples were frozen for 30 min at -80 °C, the liquid organic phase was then transferred into a new vial, and the remaining phase was then extracted for a second time as described above and pooled with the first one. Diethyl ether was allowed to evaporate over night at room temperature.

Following the manufacturer's instructions, samples were re-dissolved and diluted in assay buffer. The concentrations of all hormones (17 $\beta$ -estradiol—E2, testosterone—T, 11-ketotestosterone—11-KT, 17,20 $\beta$ -progesterone—17,20-P) were determined in duplicates using a standard dilution series. Absorption of the assays was measured with an Infinite M200 microplate reader (Tecan) at 412 nm.

### Data analysis

Data are presented as mean  $\pm$  SD of  $n$  samples. For the statistical analysis, data were analyzed for normality (Kolmogorov–Smirnov, passed if  $>0.05$ ) and homogeneity of

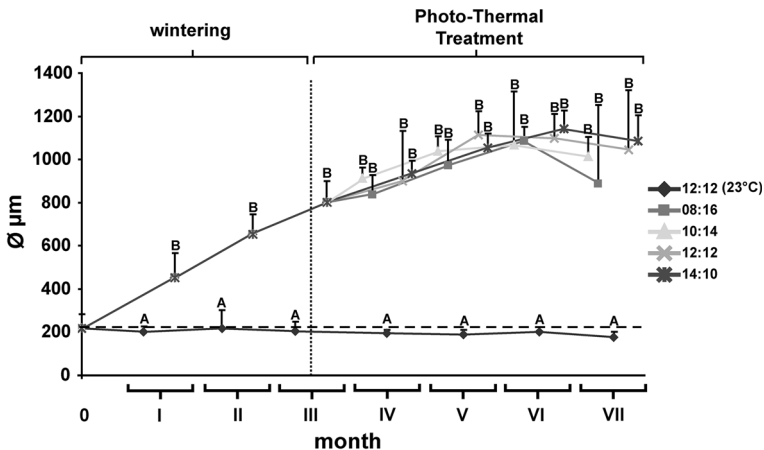


variances (log-transformed if necessary). Subsequently, parametric Tukey–Kramer multiple comparison or nonparametric Dunn’s multiple comparison test was carried out ( $p < 0.05$ ). Statistical analysis was performed using the software packages SPSS 14.0 and GraphPad Prism 4.03.

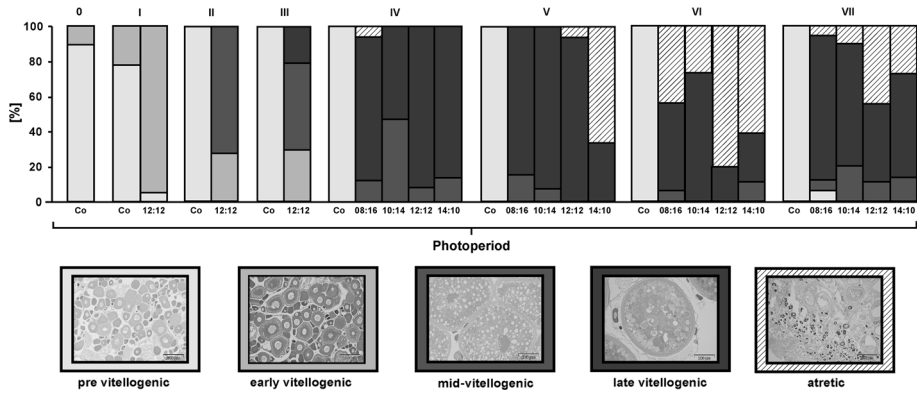
## Results

### Gonad development

In female pikeperch, 3 months of wintering and subsequent 4 months of photo-thermal treatment (PTT) induced vitellogenesis and supported maturation. This was revealed by increasing follicle diameter (Fig. 3) and ongoing ripening of the gonads, in contrast to the control fish reared continuously at 23 °C (Fig. 4). In noncontrol females, follicle diameter increased continuously from the first day of wintering onwards (mean diameter measured at day zero was  $217 \pm 65 \mu\text{m}$ ), revealing successful induction of vitellogenesis with clearly vitellogenic follicles ( $800 \pm 97 \mu\text{m}$ ) after wintering (Fig. 3). After only 3 months, at the end of the wintering phase, 29 % of early vitellogenic, 50 % of mid-vitellogenic, and 21 % of late vitellogenic follicles (LV) could be observed (Fig. 4). Thereafter, follicle growth (Fig. 3) and vitellogenesis (Fig. 4) progressed in all PTT groups, but with only minor differences between treatments: Generally, with increasing day length, higher ratios of late vitellogenic follicles were observed at an earlier time point. For example at 12:12 and 14:10, a maximum of 92 and 86 % of LV were recorded after 4 months, whereas at 08:16 and 10:14 the highest LV (85 and 93 %) was noticed one month later. Similarly, atretic follicles appeared first in the 12:12 and 14:10 treatments (except for one female at



**Fig. 3** Follicle diameter (mean ± SD) of female pikeperch after 0, I, II, III, IV, V, VI, and VII months of photo-thermal treatment (PTT) compared to control females kept at 12:12 and 23 °C. The dashed line indicates the status at day zero, the dotted line the end of wintering. The number of fish per treatment and sampling point is given in Table 1. To provide an optimal overview, data of the experimental groups at a respective sampling point are presented apart from one another, as identified on the x-axis. Significant differences between groups at a sampling point are indicated by capital letters (Tukey–Kramer/Dunn’s multiple comparison test). Significant differences within an experimental group over the experimental period are displayed in the supplemental material part (Tukey–Kramer/Dunn’s multiple comparison test)

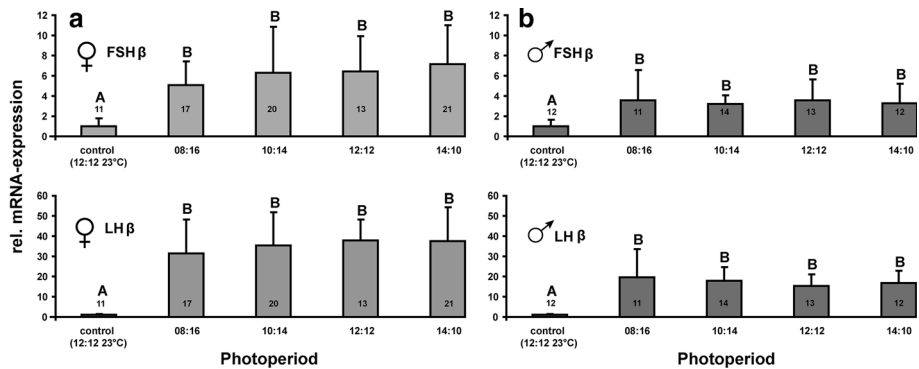


**Fig. 4** Relative abundance of follicle stages after 0, I, II, III, VI, V, VI and VII months of photo-thermal treatment (PTT) compared to control females kept at 12:12 and 23 °C. The number of fishes per treatment and sampling point is given in Table 1

08:16 after 4 months), again at a higher ratio than at shorter day lengths. Concomitant to the ongoing maturation during the PTT, the follicle diameter increased differentially, though not significant between groups (Fig. 3). Peak diameter was observed after 7 months in females of 12:12 ( $1113 \pm 110 \mu\text{m}$ ) and 14:10 ( $1142 \pm 86 \mu\text{m}$ ) treatments. Females of the short-day treatments developed only slightly smaller follicles with  $1087 \pm 64$  (8:16) and  $1067 \pm 250 \mu\text{m}$  (10:14) also after 7 months.

### Gene expression analysis

In the control fish, basic expression levels of LH $\beta$  and FSH $\beta$  in the pituitary were comparable between males and females (Fig. 5). In all PTT groups, mRNA expression of the gonadotropins LH $\beta$  and FSH $\beta$  was significantly higher than in the control. However, there were no significant differences among the PTT groups, neither in males nor in females.



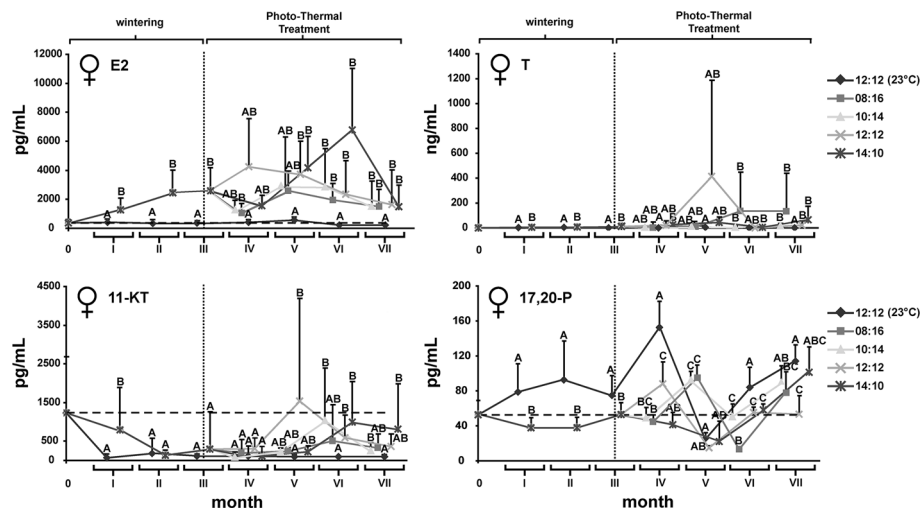
**Fig. 5** Pituitary mRNA expression (mean  $\pm$  SD) of follicle-stimulating hormone  $\beta$ -subunit (FSH $\beta$ ) and luteinizing hormone  $\beta$ -subunit (LH $\beta$ ) in female (a) and male (b) pikeperch after 4 months of photo-thermal treatment (PTT) compared to the control fish kept at 12:12 and 23 °C. The number of samples is given in Table 1 (females) and Table 2 (males). Significant differences between groups are indicated by capital letters (Tukey–Kramer/Dunn’s multiple comparison test)

After the 4 months of PTT, FSH $\beta$  exhibited a fivefold (08:16) to sevenfold (14:10) increase in females compared to control females. In males, slightly lower expression levels of FSH $\beta$  were recorded, between three- (10:14 and 14:10) and fourfold (08:16 and 12:12). The expression of the LH $\beta$  subunit was even more prominently elevated after PTT. In males, expression was between 15 (12:12) and 20 times (08:16) higher than the control. In females, the upregulation was even more pronounced with expression level between 32 (08:16) and 38 times (12:12) higher than the control females.

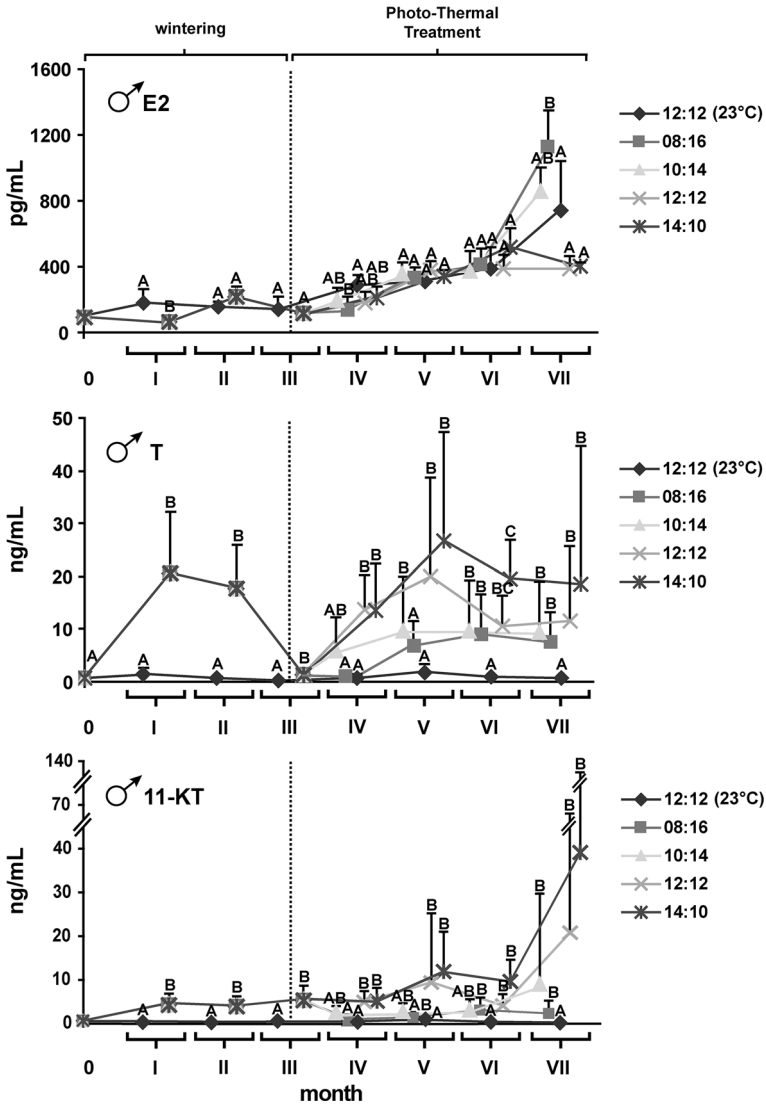
**Sex steroids**

Maturation in both sexes was correlated to the sex-specific steroids: E2 in females (Fig. 6), and 11-KT and T in males (Fig. 7) were observed in high concentrations in the PTT groups. In the control, females exhibited baseline concentrations of 220  $\pm$  50 up to 550  $\pm$  250 pg/mL E2, 0.1  $\pm$  0.1 up to 5.9  $\pm$  10.7 ng/mL T, and 110  $\pm$  40 up to 1200  $\pm$  1500 pg/mL 11-KT (Fig. 6). Males of the control also showed continuously low steroid concentrations between 100  $\pm$  40 and 750  $\pm$  300 pg/mL E2, 0.23  $\pm$  0.13 up to 1.8  $\pm$  1.5 ng/mL of T, and between 0.3  $\pm$  0.2 and 0.9  $\pm$  0.5 ng/mL of 11-KT (Fig. 7).

In females (Fig. 6), estradiol concentration already increased significantly during the wintering phase from 380  $\pm$  230 (day zero) up to 2600  $\pm$  1500 pg/mL (end of wintering after 3 months) and remained elevated in all PTT. Still, depending on the photoperiod, different peak values were observed over time. E2 decreased slightly after wintering during the first month (month four of the complete experiment, month one of the PTT) when the



**Fig. 6** Plasma concentration of sex steroids (mean  $\pm$  SD) in female pikeperch after 0, I, II, III, VI, V, VI and VII months of photo-thermal treatment (PTT) compared to control females kept at 12:12 and 23  $^{\circ}$ C. The dashed line indicates the status at day zero, the dotted line the end of wintering. The number of fish per treatment and sampling point is given in Table 1. To provide an optimal overview, data of the experimental groups at a respective sampling point are presented apart from one another, as identified on the x-axis. Significant differences between groups at a sampling point are indicated by capital letters (Tukey–Kramer/Dunn’s multiple comparison test). Significant differences within an experimental group over the experimental period are displayed in the supplemental material part (Tukey–Kramer/Dunn’s multiple comparison test). E2 17 $\beta$ -estradiol, T testosterone, 11-KT 11-ketotestosterone, 17,20-P 17,20 $\beta$ -progesterone



**Fig. 7** Plasma concentration of sex steroids (mean  $\pm$  SD) in male pikeperch after 0, I, II, III, VI, V, VI and VII months of photo-thermal treatment (PTT) compared to control males kept at 12:12 and 23 °C. The *dashed line* indicates the status at day zero, the *dotted line* the end of wintering. The number of fish per treatment and sampling point is given in Table 2. To provide an optimal overview, data of the experimental groups at a respective sampling point are presented apart from one another, as identified on the x-axis. Significant differences between groups at a sampling point are indicated by capital letters (Tukey–Kramer/Dunn’s multiple comparison test). Significant differences within an experimental group over the experimental period are displayed in the supplemental material part (Tukey–Kramer/Dunn’s multiple comparison test). E2 17 $\beta$ -estradiol, T testosterone, 11-KT 11-ketotestosterone

designated photoperiod of the experimental groups was established. Only the fish that remained at 12:12 revealed a constant increase in E2, peaking after 4 months at  $4200 \pm 3300$  pg/mL. Higher peak levels of E2 were observed with increasing day lengths:

Whereas females at the two shortest day lengths peaked at  $2600 \pm 1400$  pg/mL (08:16) and at  $2900 \pm 2500$  pg/mL (10:14), females exposed to the longest illumination time 14:10 reached a maximum concentration of  $6800 \pm 4200$  pg/mL. During wintering, T increased only up to  $7.9 \pm 12$  ng/mL. Thereafter, T increased further, but peak levels were quite different among the treatment groups:  $140 \pm 310$  (08:16, sixth month),  $17 \pm 18$  (10:14, seventh month),  $410 \pm 780$  (12:12, fifth month), and  $64 \pm 110$  ng/mL (14:10, seventh month). Interestingly, 11-KT dropped from day zero onwards ( $1200 \pm 1500$  pg/mL) and only increased thereafter, correlated to the appearance of late vitellogenic follicles in the respective PTT groups. Again, the highest level of 11-KT ( $1500 \pm 2700$  pg/mL) was observed in the group that remained at 12:12 after wintering, which was also observed earliest after a period of 5 months (2 month of PTT). In the following month, remaining treatments reached peak levels with  $510 \pm 930$  (08:16),  $1000 \pm 1400$  (10:14), and  $1000 \pm 1000$  pg/mL (14:10). Similarly, in females of the control group, 11-KT concentrations decreased, and in contrast to the PTT, groups remained low, fluctuating around  $110 \pm 40$  pg/mL. In the control females, 17,20-P constantly increased up to  $150 \pm 30$  pg/mL in the fourth month, revealing the highest concentration of all experimental groups. After peaking, 17,20-P dropped and then increased again, irrespective of the maturation of the gonad in all PTT groups as well as in the control.

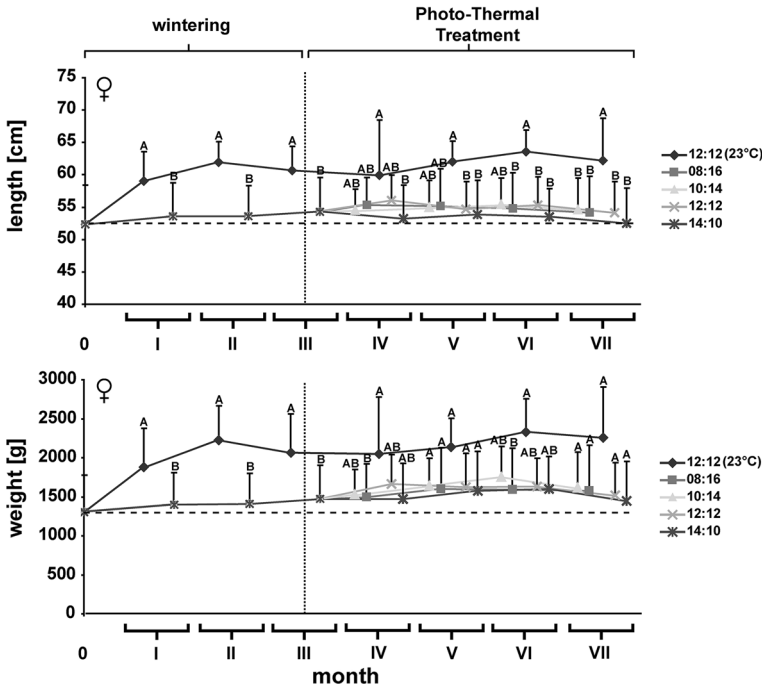
In males, T peaked at  $21 \pm 12$  ng/mL during wintering, but returned to a baseline level of  $1.5 \pm 0.5$  ng/mL after 3 months (Fig. 7). Subsequently, plasma T concentrations increased again. Here, respective peak concentrations increased with increasing day lengths from  $9.6 \pm 10.3$  ng/mL in the 08:16 group to  $27 \pm 21$  ng/mL in the 14:10 males. 11-KT concentrations increased rather constantly. Again, highest concentrations were recorded at the end of the experiment with increasing day lengths, revealing  $3.1 \pm 2.8$  ng/mL at 08:16,  $8.6 \pm 21.7$  ng/mL at 10:14,  $21 \pm 45$  ng/mL at 12:12, and  $39 \pm 91$  ng/mL at 14:10. E2 increased in all treatment groups until the end of the experiment. In contrast to the androgens, highest concentrations of E2 were recorded under short-day conditions,  $1100 \pm 230$  pg/mL at 08:16 (month seven),  $860 \pm 150$  pg/mL (month seven) at 10:14,  $400 \pm 80$  pg/mL (month six) at 12:12, and  $530 \pm 120$  pg/mL at 14:10 (month six).

## Growth

After wintering, correlated to the maturation of gonads, growth almost stagnated in females and males. No significant differences between the PTT groups were observed. In maturing females, weight and total length fluctuated around  $52 \pm 6$  and  $56 \pm 4$  cm, and wet weights ranged from  $1300 \pm 440$  to  $1730 \pm 370$  g (Fig. 8). Maturing males reached comparable values with weight and length between  $1100 \pm 260$  and  $1540 \pm 410$  g and  $49 \pm 4$  and  $54 \pm 7$  cm over the experimental period (Fig. 9). In contrast, control females and males grew moderately to maximum sizes around  $63 \pm 3$  (females) and  $62 \pm 4$  cm (males), increasing their peak body weight to  $2300 \pm 400$  (females) and  $2200 \pm 400$  g (males), respectively.

## Discussion

As demonstrated by Hermelink et al. (2011, 2013), the onset of gonad maturation (puberty) in addition to subsequent maturation is temperature-controlled in the pikeperch (*S. lucioperca*). Hence, fine-tuning of the rearing protocol will substantially improve

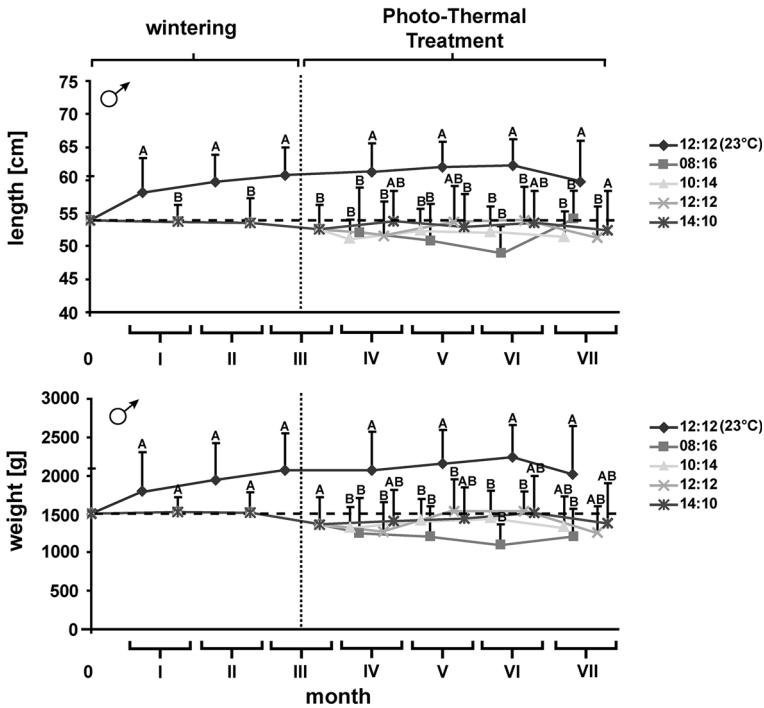


**Fig. 8** Growth (mean  $\pm$  SD) in female pikeperch after 0, I, II, III, VI, V, VI and VII months of photo-thermal treatment (PTT) compared to control females kept at 12:12 and 23 °C. The dashed line indicates the status at day zero, the dotted line the end of wintering. The number of fish per treatment and sampling point is given in Table 1. To provide an optimal overview, data of the experimental groups at a respective sampling point are presented apart from one another, as identified on the x-axis. Significant differences between groups at a sampling point are indicated by capital letters (Tukey–Kramer/Dunn’s multiple comparison test). Significant differences within an experimental group over the experimental period are displayed in the supplemental material part (Tukey–Kramer/Dunn’s multiple comparison test)

reproduction to overcome the shortage of stocking material for this emerging aquaculture species. In the Eurasian perch (*P. fluviatilis*), a closely related species, photoperiod plays a key role in the regulation of the reproductive cycle (Taranger et al. 2010; Wang et al. 2010).

The influence of the photoperiod on gonad maturation has not yet been experimentally studied in the pikeperch, although photoperiod has been examined in several studies on related species and is recently implemented by some commercial hatcheries during reproduction (Table 4). Thus, the focus of this study was to examine the influence of photoperiod on maturation in pikeperch and explore its potential for the reproductive management of pikeperch. The key hormones involved in the regulation of gonad development—E2, T and 11-KT—were assessed as well as both gonadotropins FSH and LH. This enabled a comprehensive description of the maturational progress and allowed the comparison between developmental stages. Firstly, we induced puberty as previously described (Hermelink et al. 2011) and then evaluated the influence of selected photoperiods at a temperature previously found to support optimal maturation of the gonads (Hermelink et al. 2013).

During the 3 months of wintering, vitellogenesis was successfully induced in all females. This was clearly demonstrated, firstly, by the constant follicle growth from



**Fig. 9** Growth (mean  $\pm$  SD) in male pikeperch after 0, I, II, III, VI, V, VI and VII months of photo-thermal treatment (PTT) compared to control males kept at 12:12 and 23 °C. The *dashed line* indicates the status at day zero, the *dotted line* the end of wintering. The number of fish per treatment and sampling point is given in Table 2. To provide an optimal overview, data of the experimental groups at a respective sampling point are presented apart from one another, as identified on the x-axis. Significant differences between groups at a sampling point are indicated by capital letters (Tukey–Kramer/Dunn’s multiple comparison test). Significant differences within an experimental group over the experimental period are displayed in the supplemental material part (Tukey–Kramer/Dunn’s multiple comparison test)

200  $\mu\text{m}$  to more than 1100  $\mu\text{m}$ , and secondly, by the transition to vitellogenesis and subsequent maturation. Additionally, the increase in E2 and T levels in the plasma indicated the activation of the HPG axis at the transition from previtellogenesis to vitellogenesis. Similarly in males, wintering-induced spermatogenesis correlated to increasing 11-KT blood concentrations.

Thus, at the end of the wintering period, stagnation of the gonad was overcome. In contrast, fish constantly kept at 23 °C and 12:12 confirmed complete stagnation of maturation. Only these fish exhibited substantial weight and length gain.

After the wintering phase, four different photo-thermal treatments tested whether day length influences maturation, as reported for other Perciformes such as the Eurasian perch (*P. fluviatilis*) where light has been suggested as the principal environmental cue triggering gametogenesis (Wang et al. 2006, 2010; Abdulfatah et al. 2011, 2013).

Remarkably, in female pikeperch reared at 14:10, the first appearance of atretic ovarian follicles, generally taken as evidence of spontaneous spawning (Sulistyo et al. 1998), was observed after 5 months, indicating an accelerated maturation induced by the longer day length.

**Table 4** Protocols used for the reproduction of pikeperch *S. lucioperca*, involving a wintering period to induce puberty/maturation, a maturation period and final maturation

Origin	Wintering (induction of puberty/maturation) and chilling			Ongoing maturation			Final maturation			Reference
	T (°C)	Period (d)	Photoperiod (L:D)	T (°C)	Period (d)	Photoperiod (L:D)	T (°C)	Photoperiod (L:D)		
	Domesticated	<14	min. 90	n. s.	16	n. s.	n. s.	16	n. s.	
Domesticated	<10	60–70	05:19–08:16	10–14	20–40	08:16–16:08	10–14	16:08	Landesforsch. Landwirtschaft & Fischerei Mecklenburg-Vorpommern, Institut für Fischerei, Malchower Chaussee 1, D-17194 Hohen Wangelin, Germany	
Domesticated	≤10	60	08:16	14–15	75	16:08	14–15	16:08	Institute of Inland Fisheries Potsdam-Sacrow, Im Koenigswald 2, D-14469 Potsdam, Germany/Mueller-Belecke and Zienert (2008)	
Domesticated	8	105	08:16	14	21–50	14:10	14	14:10	Mueritz AquaArt GmbH, An der Reeck 9A, D-17192 Waren, Germany	
Domesticated	n. c.	us.	n. c.	15	50	15:09	15	15:09	Fischzucht Rietschen, Forsthausweg 3A, D-02956 Rietschen, Germany	
Domesticated	4–8	98	08:16	12	24	14:10	12	14:10	Zakes (2007), Zakes and Demska-Zakes (2009)	
Domesticated/ pond	≤5	122–223	9:15/9.5:14.5/ 10:14	14–15	21	11:13/13:11/ 15:09	14–15	11:13/13:11/ 15:09	Blecha et al. (2015)	
Domesticated/ pond	n. c.	us.	n. c.	n. c.	us.	n. c.	15	14:10	Kristan et al. (2013)	
Wild	n. c.	n. c.	n. c.	7.5	us.	n. c.	16	us.	Ljubratovic et al. (2013)	
Wild	2–9	min. 84	n. c.	10–16	us.	n. c.	15–18	12:12/0:24	Ronyai (2007)	
Domesticated	8 (4)	98	08:16	12	28	14:10	12	14:10	Zakes et al. (2013)	
Wild	≤10/ 12 °C	us.	n. c.	≤10/ 12	us.	n. c.	13 + 15	12:12	Zarski et al. (2012, 2013)	

*min.* minimum, *n. c.* natural conditions, *n. s.* not specified due to confidentiality agreement, *us.* unspecified



Notably, even at the end of the experimental phase, the majority of females exposed to the shorter day lengths were still in a late vitellogenic stage with smaller follicles in average and therefore ready to undergo final maturation. Thus, an important technical note for commercial out-of-season reproduction is that shortened day length can be used to elongate the availability of potential spawners and therefore can be used to fine-tune reproduction. Furthermore, this study clearly suggests that photoperiod is only a minor factor modulating the progression of maturation, and finalization of maturation is equally achieved by temperature only.

The mRNA expression of the gonadotropin  $\beta$ -subunits was increased in both sexes and all treatment groups when compared to control fish, confirming the activation of the HPG axis and progression of gonad maturation. Nevertheless, there were no significant differences between the different day length treatments. Still, the expression levels of LH $\beta$  as well as FSH $\beta$  in females of the short-day treatments were slightly lower than those of the long-day treatments supporting the other findings here. As with our previous study, LH $\beta$  was more prominently increased compared to FSH $\beta$ . In this study, we can confirm the key role of LH toward the end of maturation as has been hypothesized in other fish species, e.g., Striped Bass (*Morone saxatilis*) (Hassin et al. 1999; Mylonas and Zohar 2001) and the European sea bass (*Dicentrarchus labrax*) (Rocha et al. 2009; Mazon et al. 2015). This suggests minor effects of the photoperiod on the expression of the gonadotropins during the mainly temperature-regulated maturation of the pikeperch.

Due to the experimental setup, the expression profiles of the gonadotropins exclusively reflect the situation at the end of the experiment. However, the repeated measurements of the sex steroids over the 4-month PTT provides a more precise insight into the influence of the photoperiod on maturation. Here, females reared under long-day conditions revealed the highest E2 concentrations, which is the crucial hormone of the female reproductive cycle. This was accompanied with a remarkable upregulation of 11-KT during the ongoing vitellogenesis (Hermelink et al. 2013). Interestingly, 11-KT is involved in the regulation of lipid (especially lipoprotein lipase) transportation during female maturation, which agrees with the results of the preceding study (Hermelink et al. 2013).

In male pikeperch, the HPG axis was activated during wintering, clearly indicated by the main androgens T as well as 11-KT. Similarly, these steroids were highest in the long-day treatments, as found with female specimens. It is well established that males of various percid fish such as the Eurasian perch (*P. fluviatilis*), yellow perch (*Perca flavescens*), white perch (*Morone americana*), and walleye (*Stizostedion vitreum*) show a clear, often biphasic, pattern of seasonal changes in the main androgens T and 11-KT, concomitant with the maturational stage (Malison et al. 1994; Jackson and Sullivan 1995; Dabrowski et al. 1996; Sulistyo et al. 2000). Less is known about the age-dependent production of the androgens. While Cierszko et al. (1998) detected similar levels of T and 11-KT in two- and three-year-old yellow perch (*P. flavescens*), the 11-KT concentrations in the three-year-old pikeperch observed during our experiment were up to three times higher at the end of the experiment than those found in two-year-old pikeperch previously reported (Hermelink et al. 2013). Yet, the highest androgen concentrations were observed in the long-day treatments and the lowest in the short-day treatments, indicating an effect of the photoperiod similarly found in females where the main estrogen E2 was highest under the long-day exposure.

In males, gonadotropin expression (in particular LH) did not reveal an effect of the photoperiod. Due to the presence of successive spermatogenic stages in the gonad, such effects are likely to have been further masked.

## Conclusion

The investigation clearly highlighted temperature and not light as the main factor to control maturation in pikeperch (*S. lucioperca*). Therefore, a clear species-specific difference in the biology of pikeperch compared to the closely related Eurasian perch (*P. fluviatilis*) is described. Nevertheless, day length appears to “fine-tune” maturation. While “long days” with an illumination time of 12 and 14 h accelerated the maturation, a delay was observed under “short days” (8 or 10 h of light). Consequently, the manipulation of the photoperiod can be implemented as a simple and cost-effective measure to elongate the availability of pikeperch spawners under commercial conditions (e.g., to elongate the availability of spawnable fish), if the optimal temperature protocol is applied concurrently. This may be particularly useful for out-of-season reproduction in RAS-based aquaculture, also with regard to endogenous rhythms that may counteract pure thermal treatment. Although the data on females are more conclusive, males of the long-day treatments were characterized by the highest concentrations of both androgens T and 11-KT, indicating that similar effects are also modulating spermatogenesis. Thus, photoperiod should not be overlooked in the future development of pikeperch aquaculture and warrants consideration in the reproductive management of pikeperch.

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