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Photoperiod affects gamete production, and protein and lipid metabolism in male narrow-clawed Crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823)

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ABSTRACT

Understanding effects of photoperiod on the reproductive efficiency and physiological responses of *Pontastacus leptodactylus* is important for successful management of the crayfish hatcheries. Spermatozoal number, gonado-somatic index (GSI), hepatosomatic index (HSI), testicular index (TI), vas deferens index (VDI), and total protein, amino and fatty acid profiles of the hepatopancreas were evaluated in male narrow-clawed crayfish *P. leptodactylus*. Animals (30–70 g) were maintained utilizing five different photoperiodic regimens (3 L:21D, 6L:18D, 24 L:0D, 0L:24D, and natural photoperiod (9 L:15D)) for 5 weeks. Different photoperiods affected spermatozoal number, GSI, and total protein, amino acid and fatty acid profiles of the hepatopancreas ($P < 0.05$). The greatest spermatozoal number and GSI were recorded in darkness ($P < 0.05$). There was the least spermatozoal number ($3.5 \times 10^6 \pm 0.5$) and GSI ($2 \pm 0.1\%$) when imposing the 9 L and 24 L photoperiodic regimens, respectively. In addition, maintenance in constant light as compared with other lighting regimens resulted in a greater total protein (43.68 ± 4.83 mg/g), phenylalanine ($7.23 \pm 0.35\%$), and total single unsaturated fatty acids (ΣMUFA) ($48.07 \pm 2.30\%$), but lesser total polyunsaturated fatty acids (ΣPUFA) ($23.36 \pm 1.07\%$), serine ($1.53 \pm 0.02\%$), Σn-6 ($11.24 \pm 1.18\%$), 20:4 (n-6) ($2.7 \pm 0.14\%$), and 20:2 ($0.67 \pm 0.17\%$) contents in the hepatopancreas ($P < 0.05$). The results of the present study indicate total darkness increases the spermatozoal production and imposing longer dark periods improved the reproductive efficiency and physical conditions in male *P. leptodactylus*.

1. Introduction

Ecological factors such as water temperature (Yazicioglu et al., 2018; Farhadi and Harlıoğlu, 2018), annual life cycle (Farhadi and Harlıoğlu, 2019), photoperiod (Harlıoglu and Duran, 2010), and nutrition (Safari et al., 2017; Safari and Paolucci, 2017) affect the reproductive efficiency of the freshwater crayfish. The photoperiodic pattern affects growth and moulting frequency (Farhadi and Jensen, 2016), survival and cannibalism (Farhadi et al., 2014) as well as reproductive efficiency (Harlıoglu and Duran, 2010) in crayfish. Photoperiod manipulation may be an effective method for improving reproductive efficiency in crayfish hatcheries (Harlıoğlu and Farhadi, 2017). Responses of crayfish to photoperiod depends on the specific environmental conditions which the species has been previously adapted (Harlıoglu and Duran, 2010; Farhadi and Harlıoğlu, 2019).

Specific photoperiodic patterns induce maturation and spawning in some decapod species (Yano, 1993; Quackenbush, 1994; Liu

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et al., 2013). Imposing longer photoperiodic regimens results in an increase in ovarian development and vitellogenin in Penaeidae and *Homarus americanus* (Yano, 1993; Quackenbush, 1994). Photoperiod requirements for reproduction, however, can vary among species. For example, *Procambarus clarkii* requires an increase in daylight for ovarian maturation, egg-laying, and spawning (Liu et al., 2013), whereas *P. leptodactylus* had a greater reproductive performance when there was a longer dark period with the photoperiodic regimen in the enclosure the animals were housed (Harlıoglu and Duran, 2010).

Freshwater crayfish are an important economic aquaculture species (Kouba et al., 2012). Understanding factors that can improve reproductive efficiency, therefore, is required for effective production of juveniles either for restoring the wild population or for aquaculture purposes (Harlıoğlu and Farhadi, 2017).

The spermatozoon in crayfish and other decapods is aflagellate and immotile (Niksirat et al., 2013a, 2013b, Kouba et al., 2015; Yazicioglu et al., 2016). In addition, male gametes in crayfish are concentrated in astructure, the spermatophore (Tudge, 2009; Niksirat et al., 2014a; Niksirat and Kouba, 2016). A sperm count in crayfish, therefore, is the precise procedure for evaluating gamete production and gamete quality after imposing different treatments in an experiment (Harlıoğlu et al., 2018).

Amino and fatty acids have important functions in the quality of crayfish spermatozoa (Harlıoğlu et al., 2013; Niksirat et al., 2016). In addition, gametes in male crayfish consist of a complex network of proteins necessary for effective reproduction (Niksirat et al., 2014b, 2015, 2016). Measurement of the fatty acids and proteins in the present experiment, therefore, may allow for a greater understanding of the effects of photoperiod on gamete production at a molecular level.

The aim of this study was to evaluate the effects of different photoperiod regimens on spermatozoal production, vas deferens index (VDI), gonado-somatic index (GSI), hepatosomatic index (HSI), testicular index (TI), total protein, amino acid and fatty acid profiles of the hepatopancreas in male narrow-clawed crayfish, the *P. leptodactylus*.

2. Materials and methods

2.1. Experimental animals

Adult *P. leptodactylus* males (n = 60; mean weight 49.1 ± 2.9 g; mean carapace length ± SE: 5.8 ± 0.09 cm) were captured from the Keban Dam Lake (Elazığ, Turkey). The animals were confined in captivity in 500 L fiberglass tanks for 2 weeks when there were natural photoperiodic conditions and fed (1.5% of the body mass) with a commercial shrimp diet (40% crude protein, 15% crude fat, 1.5% crude fiber; 2.5% calcium; 1.5% phosphorus; Beyza Feed Mill, Iran) twice a day (Safari et al., 2014; Farhadi and Jensen, 2016).

2.2. Experimental design

Crayfish were weighed and distributed into 20 plastic containers (15 L; four replicates for each treatment, three individuals per each replicate, and 12 individuals per each treatment) with a flow-through system. During the trial, water temperature, pH, and dissolved oxygen were measured daily. Mean water temperature, dissolved oxygen, and pH were 9.1 ± 0.8 °C, 7.2 ± 2 mg/L, and 7.5 ± 0.1, respectively. The treatments were designed to evaluate the effects of photoperiod on reproductive capacity of male *P. leptodactylus* were: 3 L:21D, 6L:18D, 24 L:0D, 0L:24D, and natural photoperiod (9 L:15D). For adjusting the photoperiod regimens, there were electrical plug program timers used. The study was conducted during a 5-week period. The light intensity was 270 ± 40 lx during the experiment. At the end of the trial, the animals were dissected and tissues and samples were stored at −25 °C until the analysis.

2.3. Reproductive variables and spermatozoa counting

The values for reproductive variables were determined using the procedures previously described by Harlıoğlu et al. (2012) and Harlıoğlu et al. (2013).

$$\mathrm{GSI}(\%) = \left(\frac{\text{Reproductive system wet weight}}{\text{Body wet weight}}\right)*100$$

$$\mathrm{VDI}(\%) = \left(\frac{\text{Vas deferens wet weight}}{\text{Body wet weight}}\right)*100$$

$$\mathrm{TI}(\%) = \left(\frac{\text{Testes wet weight}}{\text{Body wet weight}}\right)*100$$

$$\mathrm{HSI}(\%) = \left(\frac{\text{Hepatopancreas wet weight}}{\text{Body wet weight}}\right)*100$$

The number of spermatozoa were determined using the procedures of Farhadi et al. (2018). The number of gametes was determined using a haemocytometer attached to a light microscope (Nikon Alphaphot YS-2, Japan).

2.4. Total protein analysis

The hepatopancreas samples were washed in a physiological solution. One gram wet tissue was homogenized in cold medium with TRIS-ETDA buffer (pH = 7.4). The total protein was investigated using the method of Lowry et al. (1951). Lowry's solution (4 ml) was added to 10 µl of tissue and the solution was incubated for 10 min. There was 1/1 (v/v) water/folin reagent added. After 30 min, the absorbance of samples was determined at 750 nm. Bovine serum albumin was used as a standard.

2.5. Amino acids analysis

The amino acids were analyzed using the procedures previously described by Buch et al. (2006). The amino acids were analyzed using gas chromatography-mass spectrometry (GC–MS). For the analysis of amino acid derivatives, a Shimadzu gas chromatograph (2010 plus) modified for glass-capillary work and an FID were used. The amino acid derivatives were separated on a 20 m Supelco Slb 5 ms capillary column (Supelco, Sigma, 0.25 mm ID 0.25 mm film thickness) operating with helium as the carrier gas (45 cm/sec) with the following temperature program being utilized: increase in temperature from 120 to 150 °C at 120 °C/min (with a 5 min without temperature changes), then a further increase to 240 °C at 7 °C/min and subsequently to 285 °C at 20 °C/min (with a 18 min period of maintenance at this temperature). The temperature of the injector and detector was kept constant at 240, and 300 °C, respectively. The identification of amino acid derivatives was based on comparison of the FID chromatogram and retention times with those of a standard reference (Buch et al., 2006).

2.6. Fatty acid analysis

The lipids were extracted using hexane-isopropanol (3:2 v/v) with procedures described by Hara and Radin (1978). A Shimadzu GC-17 Ver. 3 gas chromatography (GC) (Kyoto, Japan) was used for analyzing the methyl esters. For methyl ester analysis, a 25 m long Machery-Nagel (Germany) capillary column with an inner diameter of 0.25 µm and a thickness of 25-micron film was used. The column temperature, injection temperature, and detector temperature were maintained at 120–220 C, 240 C, and 280 °C, respectively. The nitrogen carrier gas flow was 1 ml/min. The methyl esters of fatty acids were identified by comparison with external standard mixtures analyzed using the same conditions. The data were analyzed using Class GC 10 software version 2.01.

2.7. Statistical analysis

The normality and homoscedasticity of the data were assessed using Kolmogorov–Smirnov and Levene's test, respectively. The proportional data were normalized using squareroot arcsin transformation. To compare differences between different photoperiods a one-way ANOVA was utilized. Significant differences between groups were determined using the Tukey's HSD *post hoc* test in each case. The level of significance for all analyses was set at $P < 0.05$. Data were analyzed using the SPSS version 16.0 (2007 SPSS Inc.).

3. Results

There were differences ($P < 0.05$) as a result of imposing different photoperiodic regimens observed in spermatozoal number/crayfish (Fig. 1), spermatozoal number/g body weight, spermatozoal number/g vas deferens, and GSI (Table 1). The number of spermatozoa/crayfish ($1.4 \times 10^7 \pm 0.4$), spermatozoal number/g body weight ($2.8 \times 10^5 \pm 0.6$), spermatozoal number/g vas

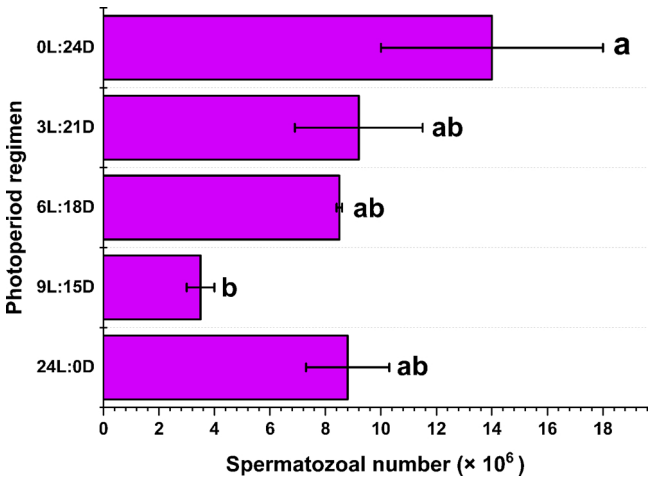

Fig. 1. Effect of photoperiod on spermatozoal number/crayfish in *P. leptodactylus*; Different letters indicate differences at P < 0.05 (mean ± SE; n = 12).

Table 1
HSI, GSI, TI, VDI, spermatozoal number/g body weight, and spermatozoal number/g vas deferens of *P. leptodactylus* in different photoperiodic regimens; Values (mean ± SE) with different letters in the columns are different (P < 0.05) (n = 12).

Treatment	HSI	GSI	TI	VDI	Spermatozoa number/g body weight	Spermatozoa number/g vas deferens
24 L:0D	3.8 ± 0.3	2 ± 0.1^{b}	0.3 ± 0.03	1.4 ± 0.06	$1.7 \times 10^{5} \pm 0.1^{ab}$	$1.1 \times 10^{7} \pm 0.1^{ab}$
9 L:15D	3.8 ± 0.3	2.4 ± 0.2^{ab}	0.3 ± 0.05	1.8 ± 0.1	$1 \times 10^{5} \pm 0.02^{b}$	$5.5 \times 10^{6} \pm 0.6^{b}$
6L:18D	4 ± 0.3	2.2 ± 0.1^{ab}	0.3 ± 0.01	1.5 ± 0.06	$1.6 \times 10^{5} \pm 0.3^{ab}$	$1 \times 10^{7} \pm 0.1^{ab}$
3 L:21D	3.8 ± 0.4	2.3 ± 0.2 ^{ab}	0.29 ± 0.03	1.6 ± 0.1	$1.8 \times 10^{5} \pm 0.4^{ab}$	$1.1 \times 10^{7} \pm 0.3^{ab}$
0L:24D	4.8 ± 0.1	2.8 ± 0.2^{a}	0.4 ± 0.06	1.8 ± 0.1	$2.8 \times 10^{5} \pm 0.6^{a}$	$1.4 \times 10^{7} \pm 0.2^{a}$

deferens ($1.4 \times 10^{7} \pm 0.2$), and GSI (2.8 ± 0.2%), were greater for the crayfish where there was imposing of the 24 h dark in comparison with the natural 9 L photoperiodic regimen ($P < 0.05$). There was the least GSI (2 ± 0.1) when there was the constant light photoperiodic regimen, however, the least spermatozoal number/crayfish ($3.5 \times 10^{6} \pm 0.5$), spermatozoal number/g body weight ($1 \times 10^{5} \pm 0.02$), and spermatozoal number/g vas deferens ($5.5 \times 10^{6} \pm 0.6$) when there was imposing of the 9 L photoperiodic regimen. There were no differences, however, observed in HSI, TI, and VDI as a result of photoperiodic regimen ($P > 0.05$; Table 1).

The results indicate photoperiod affected the total protein content of the hepatopancreas, ($P < 0.05$). For example, the total protein with the constant light photoperiodic regimen (43.68 ± 4.83 mg/g) was greater than with the other photoperiodic regimens (Fig. 2). The results also indicated that photoperiod affected the amino acid profile of the hepatopancreas ($P < 0.05$). Serine and phenylalanine were also affected by photoperiod (Table 2). There were the largest quantities of serine (3.81 ± 1.43%) and phenylalanine (7.23 ± 0.35%) with the 3 L and 24 L photoperiodic regimens, respectively.

Photoperiod affected the fatty acid profile of the hepatopancreas ($P < 0.05$). The C16:1 n-7, C18:1n-9, C18:3n-3, C20:2, arachidonic acid (C20:4n-6), total single unsaturated fatty acids (ΣMUFA), total polyunsaturated fatty acids (ΣPUFA), and Σn-6 were similar when there was imposing of all the photoperiodic regimens evaluated in this study ($P < 0.05$; Table 3; Fig. 3). With constant light photoperiodic regimen, there was the greatest ΣMUFA (48.07 ± 2.30%) and least ΣPUFA (23.36 ± 1.07%), Σn-6 (11.24 ± 1.18%), arachidonic acid (2.7 ± 0.14%), and C20:2 (0.67 ± 0.17%).

4. Discussion

4.1. Effect of photoperiod on reproductive variables

The results of the present study indicate imposition of a total darkness photoperiodic regimen could induce an increased spermatozoal production in *P. leptodactylus*. The increase of GSI (2.8 ± 0.2%) and gamete production ($1.4 \times 10^{7} \pm 0.4$) when there is a total darkness photoperiodic regimen is probably caused by the inhibitory effects of darkness on the X-organ. The X-organ sinus gland complex is a neuroendocrine organ located in the eyestalk of decapods and is the main neuroendocrine regulatory center (Alfaro-Montoya, 2010). The reproduction of male crustaceans is regulated by the function of the X-organ (Alfaro-Montoya, 2010; Alfaro-Montoya et al., 2016). Eyestalk ablation leads to improvement in sperm quality of decapod crustaceans (Vázquez-Islas et al., 2013). Furthermore, eyestalk ablation leads to a lesser concentration of moult inhibiting hormone and gonad inhibiting hormone (GIH) (Vázquez-Islas et al., 2013). Eyestalk removal leads to an increase in the storage of protein, carbohydrate, and lipid and

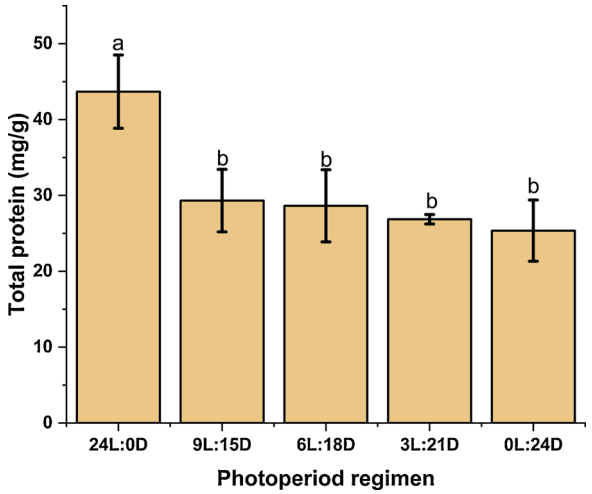

Fig. 2. Effect of photoperiod on the total protein content (mg/g) of the hepatopancreas in *P. leptodactylus*; Different letters indicate differences at P < 0.05 (mean ± SE; n = 10).

Table 2
Effect of photoperiod on the amino acids profile (%, total amino acids) of the hepatopancreas in *P. leptodactylus*; Values (mean ± SE) with different letters in the rows are different ($P < 0.05$) ($n = 10$).

Amino acids	24 L:0D	0L:24D	6L:18D	3 L:21D	9 L:15D
Arginine	0.52 ± 0.25	0.43 ± 0.21	0.19 ± 0.06	0.12 ± 0.05	0.18 ± 0.08
Alanine	7.69 ± 0.74	9.35 ± 0.52	8.53 ± 0.34	7.62 ± 0.48	8.28 ± 0.29
Glycine	7.29 ± 0.19	8.24 ± 0.20	7.46 ± 0.34	7.16 ± 0.47	7.23 ± 0.14
Valine	3.34 ± 0.30	3.00 ± 0.36	3.36 ± 0.60	5.99 ± 1.49	3.62 ± 0.58
Leucine	9.43 ± 0.36	10.05 ± 0.80	9.19 ± 0.20	8.33 ± 0.37	9.33 ± 0.35
Isoleucine	13.86 ± 0.23	13.69 ± 0.21	14.27 ± 0.53	13.77 ± 0.10	13.75 ± 0.26
Proline	7.03 ± 0.15	7.24 ± 0.34	7.08 ± 019	6.82 ± 0.14	7.13 ± 0.19
Methionine	0.68 ± 0.03	0.85 ± 0.18	1.11 ± 0.10	1.07 ± 0.18	0.91 ± 0.25
Serine	1.53 ± 0.02^{b}	1.67 ± 0.33^{b}	3.81 ± 1.43^{b}	8.81 ± 0.02^{a}	1.73 ± 0.44^{b}
Threonine	9.79 ± 0.42	7.12 ± 1.39	6.99 ± 1.03	6.29 ± 0.35	7.33 ± 1.69
Phenylalanine	7.23 ± 0.35^{a}	5.72 ± 1.88^{ab}	4.21 ± 1.97^{ab}	1.18 ± 0.50^{b}	4.22 ± 1.87^{ab}
Aspartic Acid	7.90 ± 0.29	7.09 ± 0.45	8.34 ± 0.34	7.95 ± 0.19	7.94 ± 0.28
Cysteine	12.94 ± 0.58	13.39 ± 0.29	12.65 ± 0.60	13.33 ± 0.26	12.98 ± 0.65
Glutamic Acid	3.47 ± 0.40	4.34 ± 0.07	4.46 ± 0.22	4.35 ± 0.14	3.49 ± 0.48
Lysine	1.44 ± 0.21	1.56 ± 0.16	1.20 ± 0.21	1.77 ± 0.02	1.33 ± 0.26
Histidine	0.68 ± 0.10	1.13 ± 0.7	1.43 ± 0.9	0.74 ± 0.05	0.61 ± 0.18
Tyrosine	0.67 ± 0.13	0.58 ± 0.09	0.80 ± 0.27	0.97 ± 0.07	0.69 ± 0.18
Tryptophan	3.40 ± 0.28	3.80 ± 0.20	3.61 ± 0.26	3.86 ± 0.38	3.81 ± 0.25

Table 3
Effect of photoperiod on the fatty acids profile (%, total fatty acids) of the hepatopancreas in *P. leptodactylus*; Values (mean ± SE) with different letters in the rows are different ($P < 0.05$) ($n = 10$).

Fatty acids	24 L:0D	0L:24D	6L:18D	3 L:21D	9 L:15D
C14:0	1.62 ± 0.10	1.37 ± 0.08	1.35 ± 0.11	1.38 ± 0.10	1.37 ± 0.18
C14:1	0.83 ± 0.10	0.80 ± 0.06	0.86 ± 0.01	0.78 ± 0.07	0.83 ± 0.09
C15:0	0.50 ± 0.02	0.47 ± 0.04	0.48 ± 0.02	0.45 ± 0.13	0.49 ± 0.15
C16:0	20.16 ± 0.35	19.55 ± 1.13	19.41 ± 0.44	20.68 ± 0.68	19.85 ± 1.19
C16:1 n-7	2.00 ± 0.01^{a}	1.87 ± 0.05^{a}	2.14 ± 0.08^{a}	0.96 ± 0.10^{b}	1.97 ± 0.04^{a}
C17:0	2.02 ± 0.91	1.29 ± 0.10	1.42 ± 0.11	1.61 ± 0.03	1.71 ± 0.05
C17:1	1.52 ± 0.11	1.61 ± 0.07	1.75 ± 0.05	1.50 ± 0.07	1.66 ± 0.08
C18:0	3.84 ± 0.72	3.52 ± 0.27	3.97 ± 0.20	4.45 ± 0.07	3.74 ± 0.42
C18:1n-9	28.15 ± 1.60^{ab}	28.99 ± 1.77^{a}	22.9 ± 0.83^{b}	24.84 ± 1.09^{ab}	22.8 ± 0.73^{b}
C18:2n-6	8.14 ± 0.90	8.78 ± 0.64	10.2 ± 0.89	8.32 ± 0.90	8.88 ± 0.74
C18:3n-3	4.12 ± 0.20^{b}	4.6 ± 0.15^{b}	5.77 ± 0.29^{a}	3.87 ± 0.18^{b}	4.32 ± 0.23^{b}
C20:2	0.67 ± 0.17^{b}	0.87 ± 0.09^{ab}	0.88 ± 0.04^{ab}	1.12 ± 0.03^{a}	0.89 ± 0.08^{ab}
C20:4n-6 (AA)	2.7 ± 0.14^{b}	3.07 ± 0.20^{ab}	3.11 ± 0.16^{ab}	3.45 ± 0.16^{a}	3.17 ± 0.17^{ab}
C20:5n-3 (EPA)	2.57 ± 0.17	3.19 ± 0.32	3.11 ± 0.09	3.15 ± 0.27	3.17 ± 0.25
C24:1	0.27 ± 0.03	0.20 ± 0.07	0.16 ± 0.01	0.25 ± 0.12	0.22 ± 0.09
C22:4n-6	0.46 ± 0.03	0.42 ± .05	0.45 ± 0.01	0.58 ± 0.08	0.48 ± 0.07
C22:5 n-3	2.54 ± 1.12	1.01 ± 0.12	1.22 ± 0.1	1.73 ± 0.26	1.28 ± 0.31
C22:6n-3 (DHA)	2.83 ± 0.08	2.99 ± 0.37	2.65 ± 0.12	3.3 ± 0.34	2.75 ± 0.15
ΣSFA	28.30 ± 1.17	26.59 ± 1.08	27.39 ± 0.12	28.87 ± 0.61	27.08 ± 0.58
ΣMUFA	48.07 ± 2.30^{a}	47.05 ± 0.83^{ab}	41.92 ± 0.61^{b}	44.70 ± 0.95^{ab}	45.75 ± 0.85^{ab}
ΣPUFA	23.36 ± 1.07^{b}	25.82 ± 1.48^{ab}	27.89 ± 1.11^{a}	25.80 ± 1.14^{ab}	25.83 ± 1.08^{ab}
Σn-3	11.93 ± 1.62	12.87 ± 0.87	14.30 ± 0.34	13.34 ± 0.67	12.95 ± 0.83
Σn-6	11.24 ± 1.18^{b}	16.13 ± 2.00^{ab}	19.38 ± 2.30^{a}	17.92 ± 0.24^{ab}	17.58 ± 0.25^{ab}
n-3/n-6	1.01 ± 0.23	0.85 ± 0.16	0.67 ± 0.01	0.72 ± 0.04	0.81 ± 0.09
Unidentified	0.63 ± 0.06	0.30 ± 0.18	0.74 ± 0.03	0.38 ± 0.16	0.49 ± 0.09

ΣSFA, total saturated fatty acids, ΣMUFA, total single unsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids.

suppression of the process of organic consumption in decapods. Eyestalk removal, therefore, leads to inhibition of the rate of protein and lipid catabolism and an increase of the storage of energy (Sun et al., 2014).

The reduction of GSI (2 ± 0.1%) and amount of the total protein (43.68 ± 4.83 mg/g) content of hepatopancreas with the constant light photoperiodic regimen is probably due to this stressful condition. The increase in light intensity and photoperiod also results in an increase in the quantity of haemolymph lactate, glucose and total protein in crayfish species (Fanjul-Moles et al., 1998; Lanari et al., 2011; Farhadi and Jensen, 2016). Farhadi and Jensen (2016) suggested that long photophases (18 L) elevated stress responses, as determined by an increase in haemolymph glucose, lactate, and total protein concentrations. In addition, the reduction of GSI in constant light could be due to the stimulating effects of light on the X-organ. The stimulation of this organ increases the content of GIH and suppresses gonad maturation and spermatogenesis in decapods (Alfaro-Montoya, 2010).

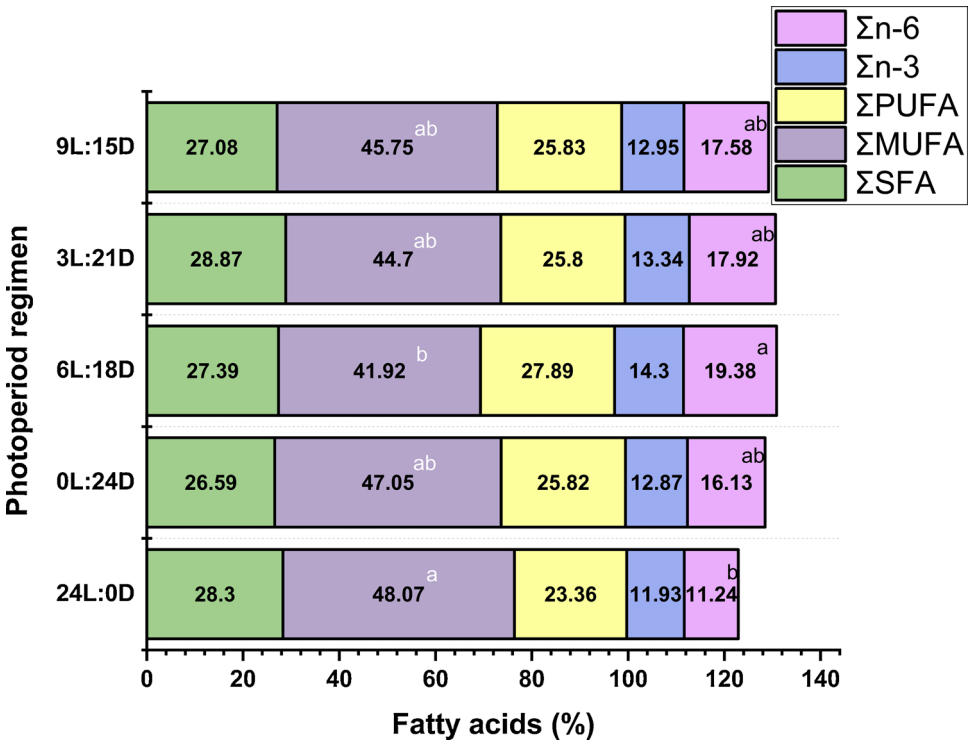

Fig. 3. The percentage of ΣSFA, ΣMUFA, ΣPUFA, Σn-3, and Σn-6 with imposing of different photoperiodic regimens in the hepatopancreas of *P. leptodactylus*; Different letters indicate differences at $P < 0.05$ (mean ± SE; n = 10).

4.2. Effect of photoperiod on protein and lipid metabolism

The hepatopancreas is the most important organ that provides substrates and transforms energy into forms that can be utilized in the spermatogenesis process (Sokolowicz et al., 2006) in decapods. These substrates are used by gametes for important biological functions such as sperm capacitation and fertilization (Niksirat et al., 2014b, 2015, 2016). Monounsaturated fatty acids (MUFA) are used as an energy supply in aquatic animals (Luo et al., 2009). Crayfish are nocturnal feeders and have lesser locomotor activity during the daylight hours (Bojsen et al., 1998). The increase of MUFA and total protein in the hepatopancreas with a constant light photoperiodic regimen may be due to lesser locomotor activity and greater energy storage when there is the photoperiod. Farhadi and Jensen (2016) reported that *P. leptodactylus* when reared in longer photoperiodic regimens had a greater lipid content than maintained with shorter photoperiodic regimens. Long photoperiodic cues lead to a decrease in the frequency of moulting in *P. leptodactylus* (Farhadi et al., 2014). Greater lipid content in reared crayfish when there are longer photophases is probably caused by decreases in moulting frequency (Farhadi and Jensen, 2016). In decapods, lipids serving as an energy source for moulting are accumulated during the intermoult stage (Lautier and Lagarrigue, 1987). Stressor substances affect the hepatopancreatic cells and biochemical composition of the hepatopancreas (Oliveira and da Silva, 2000; Pervaiz et al., 2015). Pervaiz et al. (2015) observed enlargement of the lumen of the tubules in the hepatopancreas and a greater HSI in *Macrobrachium dayanum* when there was a constant light photoperiodic regimen.

Fatty acids such as highly unsaturated fatty acids (HUFAs), eicosapentaenoic acid (EPA), PUFA, docosahexaenoic acid (DHA), n-3, and arachidonic acid are important for testicular maturation in decapods (Siangcham et al., 2015). Harlıoğlu et al. (2013) reported that dietary n-3 series fatty acids led to an increase in the number of spermatozoa in *P. leptodactylus*. The reduction of spermatozoal production and GSI when there is a constant light photoperiodic regimen is probably caused by a reduction of ΣPUFA and arachidonic acid.

The metabolism of amino acids and proteins in decapods when there are different environmental factors and stressors prevalent is not clear. The increase in total protein in the hepatopancreas of *P. leptodactylus* when there is a constant light photoperiodic regimen may be due to the accumulation of amino acids as a result of this regimen. There have been suggestions that there is deamination of the released amino acids from the various parts of the body affected by hypo-osmotic stress in the hepatopancreas, and that carbon chains of amino acids are utilized as a substrate for the gluconeogenic pathway in the hepatopancreas (Rosas et al., 2001; Chittó et al., 2009). An increase in the hepatopancreatic phosphoenolpyruvate carboxykinase activity was observed in the shrimp, *Penaeus vannamei*, acclimated to conditions of relatively lesser, as compared with what is normal, salinity by Rosas et al. (2001). Proteins are an important element of the male gametes in crayfish (Niksirat et al., 2014b, 2015). Any disturbances in the function of the hepatopancreas, as a supplier of materials to the gonads, may affect the quality of gamete production in these animals.

5. Conclusion

The results of this study indicate constant darkness regimen of managing lighting resulted in an enhanced sperm production and GSI in *P. leptodactylus*. Further studies are required to evaluate the effects of photoperiod on spermatogenesis and the concentration of sexual hormones in the haemolymph during the reproductive season of freshwater crayfish species. In addition, future studies are needed to explore the modifications and changes in the protein and lipid metabolism of decapods when there are stressful conditions for these animals and different environmental factors such as different photoperiodic regimens.

Declaration of Competing Interest

The authors declare no conflict of interest.

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