

1 **Long-term effects of the larval photoperiod on the subsequent growth of shi**
2 **drum *Umbrina cirrosa* L. specimens and the fillet texture at commercial size**

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17

18 **Abstract**

19 Three groups of shi drum *Umbrina cirrosa* L. were reared with different photoperiod regimes: 24L, 12L:12D and
20 16L:8D (natural photoperiod) during the larval period and then all of them were transferred to a natural photoperiod. At 11.8
21 and 20 months of age, the body growth and the muscle parameters reached the highest values in the 24L and 12L:12D
22 groups. The 16L:8D group showed the lowest growth. When comparing 24L with 12L:12D, the highest number of white
23 fibres was found in the 24L group, whereas the greatest fibres size was reached in the 12L:12D group.

24 Commercial size (28-30 cm; 290-340 g) was reached at 20 months of age in the 24L and 12L:12D groups, but at 23
25 months in the 16L:8D group. When comparing the three groups at the commercial stage, the larval photoperiod effect was
26 still observed, such that the highest fibres number was again found in the 24L group, whereas the greatest fibres size was
27 reached in the 12L:12D group. The highest values of textural hardness were observed in the 16L:8D and 24L groups. A
28 nutritional analysis was carried out in the 16L:8D group, which showed the following percentage values: 2.66, 21.2, 74.4,
29 and 1.46 of fat, protein, moisture and ash, respectively.

30

31 **Keywords:** photoperiod; muscle cellularity; growth; texture.

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33

34 **Introduction**

35 The growth of the skeletal muscle involves the recruitment of stem cells and subsequent hypertrophy of
36 muscle fibres (Weatherley *et al.*, 1988). The relative contribution of muscle fibre hypertrophy and hyperplasia to
37 the total muscle growth varies according to endogenous and exogenous factors. Some of the most important
38 external factors are temperature (Johnston, 1999; Johnston *et al.*, 1998; 2011; Ayala *et al.*, 2000a, 2001a,b, 2003;
39 López Albors *et al.*, 2003; García de la Serrana *et al.*, 2012; Campos *et al.*, 2013a,b), the photoperiod (Johnston
40 *et al.*, 2003a, 2004), exercise training (Johnston and Moon, 1980), and diets (Weatherley *et al.*, 1980;
41 Fauconneau *et al.*, 1997).

42 Photoperiod has been shown to affect sexual maturation, locomotor activity, smolting and growth in
43 some species (Purchase *et al.*, 2000). Long and continuous photoperiods can stimulate feed efficiency and
44 enhance the growth in different species, like Atlantic salmon *Salmo salar* L. (Johnston *et al.*, 2003a, 2004), sea
45 bream *Pagrus major* (Temminck and Schlegel, 1843) (Biswas *et al.*, 2005) and Atlantic halibut *Hippoglossus*
46 *hippoglossus* L. (Simensen *et al.*, 2000). In cod *Gadus morhua* L., Nagasawa *et al.* (2012) found that the mean
47 weight of juveniles reared under continuous light was 13% greater than those kept under a natural photoperiod
48 for 120 days. These authors studied the molecular mechanisms of photic-induced plasticity of muscle growth and
49 found changes in expression of the genes involved in epigenetic regulation. Other authors (Lazado *et al.*, 2014)
50 studied the myosin gene transcription in fast skeletal muscle of Atlantic cod and found that continuous light
51 elevated mRNA levels of several myosins in muscle when compared to a natural photoperiod.

52 The shi drum *Umbrina cirrosa* L. is a member of the Sciaenidae family. This species is a good
53 candidate for Mediterranean aquaculture because of its high growth rate, adaptability to culture conditions and
54 high market price (Mylonas *et al.*, 2004). In a previous work, Ayala *et al.* (2013) studied the larval growth of shi
55 drum under different photoperiod regimes, finding a significant effect of light regime on muscle and body
56 growth during the larval and early postlarval stages. However, it is unknown whether the larval photoperiod
57 effect persists in more advanced life stages of this species, as found in salmon by Johnston *et al.* (2003a, 2004).
58 These authors observed a long-term effect of the early photoperiod on the muscle cellularity of adult specimens
59 of salmon. In commercial size specimens, the muscle fibre density was higher on salmon previously kept with
60 continuous light resulting in firmer flesh (Johnston *et al.*, 2004). Similarly, in cod, Imsland *et al.* (2007) found
61 that short-term environmental manipulation during the early juvenile stage had a large impact on harvesting size
62 of nearly 3 years later. In turbot *Scophthalmus maximus* L., Imsland *et al.* (2013) studied the long-term effect of

63 the photoperiod and found that long term rearing in continuous light reduced growth, whereas short term
64 exposure to continuous light stimulated growth since the greatest final mean weights were reached in groups
65 previously kept in short phases of 24L and then transferred to 16L:8D until harvest.

66 In order to determine whether the early photoperiod influences the growth of shi drum in more
67 advanced life stages, all the larval groups previously studied by us in this species (Ayala *et al.*, 2013) were
68 transferred to an ambient photoperiod after larval metamorphosis and kept in separate tanks until reaching
69 commercial size. Also, textural values were analyzed at the end of the experiment (commercial size), to
70 determine whether muscle cellularity influences the firmness of flesh in this species, as found in other species
71 (Hatae *et al.*, 1990; Hurling *et al.*, 1996; Johnston *et al.*, 2000a, 2004; Periago *et al.*, 2005; Ayala *et al.*, 2010).
72 The results of this study may be of great interest to aquaculture since the manipulation of the photoperiod during
73 the larval stage can not only accelerate growth during this early phase, but also to maintain these effects into the
74 adult stages, leading to greater production at commercial size stages.

75

76 **Material and Methods**

77 This experiment was carried out with specimens of shi drum from a stock of spawners adapted to
78 captivity at the Instituto Español de Oceanografía (Centro Oceanográfico de Murcia, Mazarrón, Spain). The
79 rearing conditions of the specimens (eggs, larvae and postlarvae) were described in a previous study (Ayala *et al.*
80 *et al.*, 2013). Briefly, 547,000 fertilized eggs were placed in one cylindrical, 1 m³ capacity glass-fibre tank, with
81 continuous light and \pm 21.5 °C. Newly hatched larvae were divided into three groups. Each group was placed in
82 two cylindrical, 0.5m³ capacity glass-fibre larva cultivation tanks, with a density of 30 larvae/litre (15,000
83 larvae/tank). The three experimental groups were maintained under the following photoperiods from hatching
84 until the end of the larval metamorphosis: 24-h light/0-h dark (24L) (continuous light); 16-h light/8-h dark
85 (16L:8D) (natural light) and 12-h light/12-h dark (12L:12D). After the larval metamorphosis (July 2011), the
86 specimens were transferred to a natural photoperiod (\pm 16L:8D) into 2 m³ capacity glass-fibre square tanks until
87 the end of the experiment (20-23 months of age). At the beginning of the experiment the density of the fish was
88 \pm 10 kg/m³ in both the 24L and 12L:12D groups, but it was \pm 13.25 kg/m³ in the 16L:8D group. In the
89 subsequent stages the density was \pm 20 kg/m³ in the 24L and 12L:12D groups, but it was \pm 30 kg/m³ in the
90 16L:8D group. According to Collet (2007), the stocking density in the range of 10-50 kg/m³ is optimal and
91 results in similar growth in juveniles of this species. The fish were fed *ad libitum*. The composition of the feed
92 was: 47 % protein, 20 % fat, 7.7 % ashes, 2.5 % cellulose, 1.4 % phosphorous. The trading company for the feed

93 was Skretting (Spain). The sampling points were carried out at 11.8 months of age (in June 2012), at 20 months
94 (in January 2013, which coincided with the commercial size in the 24L and 12L:12D groups), and at 23 months
95 in the 16L:8D group (commercial size in this group, in April 2013). The temperature used in this experiment
96 corresponded with the natural temperature of the sea, such that in June 2012 (first sampling point) it was
97 increasing gradually from 21.5 to 26 °C. In the subsequent sampling points, the temperature was \acute{e} 14-15 °C in
98 January 2013 and ranged between 14.5 and 18 °C in April 2013. Survival was \acute{e} 100 % in all the tanks.

99 At each sampling point, 8-10 specimens from each light regime were randomly chosen, slaughtered by
100 clove oil anesthesia and then delivered to the veterinary faculty of Murcia.

101

102 **Quantitative analysis of body and muscle growth**

103 Total body length and body weight were measured in all specimens. Also, the eviscerated body weight
104 was measured at 20-23 months of age. After measuring these body parameters, the samples were cut transversely
105 to the long body axis and whole body slices of 5 mm thickness were obtained. Then, whole cross muscle
106 sections from each fish were photographed for measurement by a morphometric analysis system (Sygma-Scan
107 Pro_5). Subsequently, these body slices were cut into smaller blocks and then snap frozen in 2-methylbutane
108 over liquid nitrogen. Later, sections of 8 μm thickness were obtained from those frozen blocks in a cryostat
109 (Leyca CM 1850) and stained with haematoxylin-eosin for morphometric studies.

110 Muscle growth was quantified by means of the morphometric analysis system cited above. The
111 following parameters were measured: total cross-sectional area of the red and white muscles; number of white
112 muscle fibres; size (area, minimum diameter and minor axis length) of white muscle fibres and muscle fibre
113 density (number of white fibres/ μm^2). The average size was estimated from \acute{e} 500 fibres (\pm 10 SD) located at the
114 intermediate and the apical sectors of the epaxial quadrant of the transversal section of the myotome.

115

116 **Texture profile analysis (TPA)**

117 TPA was measured at commercial size in all the fish within 24 hours after their collection. The samples
118 were obtained from the dorsal musculature on the left side of each specimen using a texture analyzer (Brookfield
119 QTS-25, CNS Farnell, Borehamwood, Hertfordshire, England) equipped with Texture Pro v. 2.1 software. The
120 test conditions involved two consecutive cycles of 50% compression with 5 s between cycles. Measurements
121 were taken with a flat-ended 20 mm diameter cylindrical probe. The crosshead moved at a constant speed of 50
122 mm/min. From the resulting force \acute{o} time curve, the following parameters were determined: hardness (N)

123 (maximum force required to compress the sample); cohesiveness (extent to which the sample could be deformed
124 prior to rupture); springiness (cm) (ability of sample to recover its original form after the deforming force is
125 removed); gumminess (N/cm²) (the force needed to disintegrate a semisolid sample to a steady state of
126 swallowing (hardness x cohesiveness); chewiness (N/cm) (the work needed to chew a solid sample to a steady
127 state of swallowing (springiness x gumminess); adhesiveness (N/s) (work necessary to overcome the attractive
128 forces between the surface of the food and the surface of the other materials with which the food comes in
129 contact). All these parameters were calculated according to Bourne (1978). The measurements were done at
130 room temperature (22±23 °C) and samples were brought to temperature 30 min before the texture profiles
131 analyses (TPA) were started.

132

133 **Proximate composition**

134 A nutritional analysis was carried out in the 16L:8D group. This group was reared under natural
135 environmental conditions and represents fish usually found in the market. In subsequent studies, it will be
136 necessary to study the nutritional composition in fish reared under different environmental conditions.

137 The flesh of dorsal and ventral fillets without skin and bones were homogenized after texture
138 measurements in an Omni_Mixer (Omni International, Waterbury, CT) to obtain a homogenous sample. Samples
139 were analyzed in triplicate for moisture, total fat, ash and total nitrogen content according to AOAC methods
140 (1999). Total protein was calculated from Kjeldahl nitrogen analysis, using a 6.25 conversion factor.

141

142 **Statistical Analysis**

143 The statistical analysis was performed with SPSS 15.0 software. The mean and standard error of the
144 mean (SEM) from each group of data were calculated. Data distribution was analyzed in each stage by the
145 Shapiro-Wilk test for $P < 0.05$. Data showed a normal distribution ($P > 0.05$). Analysis of variance (ANOVA)
146 was used to evaluate the effect of the photoperiod at each sampling point, for $P < 0.05$. Tukey's test was used to
147 compare means as post-hoc analysis.

148

149 **Results**

150 **Muscle parameters and body growth**

151 *11.8-month-old specimens (356 days posthatching)*

152

153 At this stage, the body growth was significantly greater in the 12L:12D and the 24L groups than in the
154 16L:8D group (Table 1).

155 In relation to the muscle parameters, the highest values of the transverse area of the white and red
156 muscles were reached in the 24L group, followed by 12L:12D, whereas the 16L:8D group showed the lowest
157 value (Tables 2, 3). However, these differences were only significant when comparing the 16L:8D group with
158 the other groups.

159 The number of white muscle fibres showed similar results to those described for the transverse area of
160 the myotome, such that it was higher in the 24L group, followed by the 12L:12D and the 16L:8D groups (Table
161 2; Fig. 1a,b). In the case of the muscle fibre density values, they were higher in the 16L:8D group, followed by
162 the 24L and the 12L:12D groups. The white muscle fibres size was greater in the 12L:12D group, followed by
163 the 24L group, whereas the 16L:8D group showed the lowest muscle fibres size.

164

165 *20-month-old specimens (commercial size of the 24L and 12L:12D groups)*

166 At this stage, the 24L and 12L:12D groups reached commercial size: \approx 28-30 cm total length, \approx 290-340
167 g total weight (Table 1). In contrast, the body growth and eviscerated body weight of the 16L:8D group was
168 significantly lower than in the other groups.

169 The muscle parameters showed a similar tendency to those described in the previous stage. Thus, the
170 transverse area of the white muscle and the number of white fibres were greater in the 24L group, followed by
171 the 12L:12D group, showing the 16L:8D group to have the lowest value (Table 2; Fig. 1 c,d). The muscle fibre
172 density was higher in the 16L:8D group, followed by 24L and 12L:12D ($P < 0.05$). The greatest white muscle
173 fibres size was reached in the 12L:12D group, followed by 24L and 16L:8D. In relation to the transverse area of
174 the red muscle, it was also smaller in the 16L:8D group than in the other groups, although the differences found
175 in this parameter were not significant (Table 3).

176 When comparing the body and muscle growth reached in this stage in relation to the previous stage, we
177 can observe that both body length and body weight increased significantly in the three photoperiod groups. Also,
178 all the muscle parameters grew significantly in the three photoperiod groups, except the red muscle of the 24L
179 group and the number of white fibres of the 12L:12D group, where the hyperplasia was not significant.

180

181 *23-month-old specimens: commercial size in the 16L:8D group*

182

183 At this stage, all the body parameters increased significantly in the 16L:8D group, reaching commercial
184 size, with no significant differences with respect to the body values reached by the 24L and 12L:12D groups at
185 20 months of age (Table 1).

186 Muscle parameters of 23-month-old specimens of 16L:8D were compared with the muscle parameters
187 of 20-month-old specimens of the 12L:12D and 24L groups in order to determine whether the muscle cellularity
188 was similar among the three groups at commercial size. The results showed that the transverse area of the white
189 muscle was greater in the 24L groups, followed by the 12L:12D and the 16L:8D groups, but these differences
190 were not significant (Table 2). The number and the white muscle fibres density was also higher in the 24L group,
191 followed by the 16L:8D group, showing the 12L:12D group to have the lowest values, although such differences
192 were not significant either. In contrast, the white muscle fibres size was significantly greater in the 12L:12D
193 group than in the other groups. When comparing the 24L with the 16L:8D group, the muscle fibres size did not
194 show significant differences.

195

196 **Textural and Nutritional parameters**

197 Textural parameters were measured in all the groups at commercial size (20 months in the 24L and
198 12L:12D groups *versus* 23 months in the 16L:8D group). At this commercial stage, the textural values only
199 showed significant differences for the hardness and adhesiveness parameters (Table 4). The hardness values
200 were higher in the 16L:8D group, followed by 24L, showing the 12L:12D group to have the lowest values.

201 In order to determine whether the textural parameters were correlated with the muscle parameters, a
202 correlation analysis was carried out between them, by attaching the data from the three photoperiod groups. This
203 analysis showed a slight negative correlation among the muscle fibres size and most of the textural parameters
204 (firmness, gumminess, adhesiveness, chewiness and springiness), but this correlation was not significant ($P >$
205 0.05). On the contrary, a positive correlation was found among the number and the density of the muscle fibres
206 and most of the textural parameters, but this correlation was only significant when correlating the number of
207 fibres with the gumminess and the cohesiveness parameters.

208 The nutritional analysis was carried out in the 16L:8D group (natural photoperiod). The results are
209 shown in Table V, where they are compared with the results found in this species by other authors. Our values
210 show a relatively low fat level (2.6%), corresponding to lean species belonging to the Sciaenidae family.
211 Similarly, the rest of parameters (Table 5) are within the normal levels in sciaenids fish.

212

213 **Discussion**

214 Fish muscle is plastic in its response to environmental conditions: temperature, photoperiod, salinity,
215 etc. Hence, the external factors influences the number and size of red and white muscle fibres (Johnston, 1999;
216 Ayala *et al.*, 2003; López-Albors *et al.*, 2003; Johnston *et al.*, 2000a,b, 2003a,b, 2004, 2011; Campos *et al.*,
217 2013a). This influence can persist and produce long-term effects (Ayala *et al.*, 2001a; Johnston *et al.*, 1998,
218 2003a, 2004; Imsland *et al.*, 2007; Steinbacher *et al.*, 2011; García de la Serrana *et al.*, 2012; Campos *et al.*,
219 2013b).

220 In the present study, the 11.8-month-old specimens maintained with continuous light during the larval
221 period (24L group) showed the highest values of both the hyperplasia and the transverse area of the myotome,
222 followed by the 12L:12D group, showing the 16L:8D (natural photoperiod) to have the lowest values of these
223 parameters. These results are similar to those found in previous studies of the larval and early postlarval phases
224 of these experimental groups (Ayala *et al.*, 2013), and show that the larval photoperiod effect persists in more
225 advanced age stages of this species.

226 The results also showed that the continuous light photoperiod promoted hyperplasia, which coincides
227 with previous results in this species (Ayala *et al.*, 2013) and in salmon (Johnston *et al.*, 2003a, 2004). In this
228 latter species, Johnston *et al.* (2003a, 2004) maintained salmons with 24L and natural photoperiods for a short
229 time and later both experimental groups were transferred to a natural photoperiod. Four months after transferring
230 the salmons to a natural photoperiod, Johnston *et al.* (2003a) found the highest hyperplasia values in the 24L
231 group. Similarly, two weeks after transferring the salmons to a natural photoperiod, Johnston *et al.* (2004)
232 observed that the number of fast muscle fibres and the hypertrophy was higher at 24L, with the effect more
233 marked on the number than on the size of the fibres.

234 In the present study, the hypertrophy was higher in the 12L:12D than in the other groups, which shows
235 that the shorter photoperiod promotes this muscle parameter, as found previously in larva and postlarval stages
236 of this species (Ayala *et al.*, 2013).

237 Body growth (length and weight) at 11.8 months was similar between the 24L and 12L:12D groups of
238 the present study, which differs from the results found in the early phase (Ayala *et al.*, 2013), where the 24L
239 group showed significantly more growth than the other groups. The greater growth of the 12L:12D group in the
240 present study compared to that of the previous experiment shows a compensatory growth in the advanced stages
241 of this group in comparison with the early stages. In contrast, the 16L:8D group did not reach the size of the
242 other groups. In salmon, the body weight was also higher in the 24L group than in the natural photoperiod group

243 four months and two weeks after transferring the salmon to natural photoperiod (Johnston *et al.*, 2003b, 2004,
244 respectively). In two groups of cod reared at 24L and at a natural photoperiod for 120 days, Nagasawa *et al.*
245 (2012) observed that the muscle growth of the 24L group was greater not only at 120 days, but also two months
246 after the two groups were transferred to a natural photoperiod (at 180 days). These authors studied the molecular
247 mechanisms of photic-induced plasticity of muscle growth. According to their results, the lasting effects of the
248 photoperiod indicated an epigenetic transcriptional memory that could be due to chromatin remodelling that
249 occurred during the first four months in response to photoperiod changes (Nagasawa *et al.*, 2012).

250 Commercial size (é 28-30 cm, é 290-340 g) was reached at 20 months in the 24L and 12L:12D
251 specimens, whereas the 16L:8D group reached this commercial size stage three months later (at 23 months of
252 age). The results found at commercial size differ from those found in the salmon study (Johnston *et al.*, 2004).
253 These authors observed that the natural photoperiod group of salmon grew faster than the 24L group from 6-9
254 months after seawater transfer until harvest (commercial size), such that both groups reached commercial size at
255 the same age. Also, the mean weights were similar at the end of the trial in both groups of salmon. In cod,
256 Imsland *et al.* (2007) found that the juveniles reared under continuous light during the initial three month period
257 and then transferred to sea pens resulted in 1-9% larger size at harvesting compared to fish reared at a stimulated
258 natural photoperiod.

259 When comparing the muscle cellularity of the three groups at commercial size (20 months in 24L and
260 12L:12D groups *versus* 23 months in 16L:8D group) we observed that the hyperplasia and the muscle fibres
261 density was greater in the 24L group, whereas the 12L:12D group showed the highest hypertrophy values. These
262 results are similar to those found in the previous stages of the present and the preceding works on shi drum
263 (Ayala *et al.*, 2013), showing a long-term effect of the larval photoperiod on muscle cellularity in subsequent life
264 stages. Similarly, Johnston *et al.* (2004) observed a persistent effect of early light regime on muscle cellularity in
265 commercial size salmon, such that the size distribution of fibres differed between the different light regimes
266 groups, showing the 24L group to have the highest fibre density. In turbot, Imsland *et al.* (2013) studied the
267 long-term effect of photoperiod manipulation for 46 months and found that the short-term exposure to 24L
268 stimulated growth, whereas the long-term rearing at 24L reduced growth. Thus, at harvest, the greatest mean
269 weights were reached in the group previously kept to short-term exposure at 24L and then transferred to 16L:8D
270 until harvest.

271 In relation to the textural parameters, most groups showed a positive correlation with muscle fibres
272 density, although this correlation was hardly ever significant. Johnston *et al.* (2004) found similar results, such

273 that the highest fibre density of the 24L group was accompanied by firmer flesh. This correlation between
274 muscle fibre density and flesh firmness has also been observed in other fish species (Hatae *et al.*, 1984, 1990;
275 Hurling *et al.*, 1996; Periago *et al.*, 2005). These results show that the early photoperiod effects observed in the
276 larval phase of shi drum (Ayala *et al.*, 2013) persist in subsequent life stages and influences the characteristics of
277 the flesh, in particular the texture. In turbot, Imsland *et al.* (2013) found that photoperiod had only a minor effect
278 on textural and flesh quality. However, these authors found a tendency towards higher texture shear force and
279 hardness in the 24L group.

280 In relation to nutritional composition, studies of shi drum are scarce. In our study, the fat levels were
281 relatively low, similar to those found in other sciaenids, like the meagre *Argyrosomus regius* (Asso, 1801)
282 (Piccolo *et al.*, 2006). The protein levels in the shi drum in our study were also similar to those found in meagre
283 by the cited authors. Our results also agree with those obtained by Segato *et al.* (2007) from the dorsal fillet, with
284 the exception of the fat content, which was higher in our samples from both the dorsal and ventral fillets. This
285 could be due to the fact that the lipid content of farmed fish flesh is significantly higher in ventral than in dorsal
286 fillets, as demonstrated by Testi *et al.* (2006). From a nutritional point of view, we have considered that the
287 proximal composition analysis in a homogenized portion of the whole flesh, including both dorsal and ventral
288 areas as an edible portion of fish, gives better information about the total composition than the commonly used
289 dorsal area.

290 On the other hand, our results showed a higher content of moisture and protein and lower total fat and
291 ash content than those obtained by Segato *et al.* (2006) and Zafer *et al.* (2012) for whole-body of shi drum.
292 These results may be explained because the total fat in our study did not include viscera, like the liver which has
293 a high fat content. Furthermore, we used fillets without skin and bones, so the ash content is lower than in the
294 case of whole-body samples. However, other factors, like diet, can also influence the differences found among
295 the cited works. Alasalvar *et al.* (2002) and Orban *et al.* (2002) described that farmed fish show higher fat and
296 lower moisture than wild specimens, due to high dietary fat level in the feed and reduced activity, whereas
297 protein is considered to be a stable component of the fish body in respect to diet and feeding level depending
298 mainly on fish weight (Shearer, 1994). Also, the chemical composition can vary depending on age, size, sex
299 environment and season (Silva and Chamul, 2000).

300 Since the nutritional analysis was only carried out in the 16L:8D group, it is not possible to know
301 whether the photoperiod regime influenced these quality parameters in the other groups or no. Further studies

302 would be necessary to determinate if this environmental factor influences in the physicochemical composition of
303 this species.

304

305

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308

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427 **Table 1.** Mean values (\pm SEM) of the total length and the body weight. Different superscripts indicate significant
 428 differences ($P < 0.05$) among light regimes within each stage.

Sampling point	Photoperiod regime	Total body length (cm)	Total body weight (g)	Eviscerated weight body (g)
11.8 months	16L:8D	16 ^a (0.65)	53.12 ^a (6.94)	
	24L	22.15 ^b (1)	142.35 ^b (18.56)	
	12L:12D	23.3 ^b (1.124)	156.47 ^b (24.07)	
0 months	16L:8D	21.84 ^a (0.78)	150 ^a (25.92)	132.5 ^a (22.81)
(commercial size in 24L and 12L:12D specimens)	24L	30.75 ^b (0.79)	343.75 ^b (29.74)	325.69 ^b (27.34)
	12L:12D	28.69 ^b (0.91)	291.61 ^b (32.54)	260.63 ^b (28.76)
23 months	16L:8D	28.69 (0.38)	306.62 (6.03)	276.88 (6.87)
(commercial size in 16L:8D specimens)				

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430 **Table 2.** Mean values (\pm SEM) of the white muscle of shi drum. Different superscripts indicate significant differences (P
 431 < 0.05) among light regimes within each sampling point. White fibres density: white muscle fibres density (number of
 432 fibres/ μm^2) $\times 10^5$

Sampling point	Photoperiod regime	Transverse area of the White muscle (mm^2)	White muscle fibres minor axis length (μm)	Number of white muscle fibres	White fibres density
11.8 months	16L:8D	281.16 ^a (31.66)	45.91 ^a (1.29)	112542.36 ^a (9458.39)	39.3 ^a (0.18)
	24L	566.44 ^b (44.49)	50.18 ^{ab} (1.28)	181046.77 ^b (10213.72)	31.8 ^b (0.11)
	12L:12D	543.98 ^b (56.19)	53.55 ^b (2.27)	167566.27 ^{ab} (23661.5)	30 ^b (0.19)
20 months (commercial size in 24L and 12L:12D specimens)	16L:8D	570.38 ^a (61.49)	51.5 ^a (1.33)	171519.94 ^a (15197.75)	27.22 ^a (1.91)
	24L	995.42 ^b (63.98)	55.78 ^b (0.63)	248389.63 ^b (16589.3)	25.1 ^a (1.38)
	12L:12D	920.44 ^b (95.78)	61.23 ^c (0.74)	209467.42 ^{ab} (21948.98)	21.6 ^a (1.25)
23 months (commercial size in 16L:8D specimens)	16L:8D	875.36 (30.77)	56.89 (0.48)	212079.45 (14140.07)	24.52 (2.06)

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436 **Table 3.** Mean values (\pm SEM) of the red muscle of shi drum. Different superscripts indicate significant differences ($P <$
437 0.05) among light regimes for each sampling point.

Sampling point	Photoperiod regime	Transverse area of the red muscle (mm ²)
11.8 months	16L:8D	10.39 ^a (0.98)
	24L	35.40 ^b (3.98)
	12L:12D	23.91 ^b (4.42)
20 months (commercial size in 24L and 12L:12D specimens)	16L:8D	25.51 ^a (7.37)
	24L	36.94 ^a (2.99)
	12L:12D	37.77 ^a (2.47)
23 months (commercial size in 16L:8D specimens)	16L:8D	41.82 (3.72)

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441 **Table 4.** Mean values (\pm SEM) of the textural values at commercial size (20-23 months). Different superscripts indicate
442 significant differences ($P < 0.05$) among light regimes.

Photoperiod regime	Hardness (N)	Gumminess (N)	Adhesiveness (N/s)	Cohesiveness (ratio)	Chewiness (N/mm)	Springiness (mm)
16L:8D	22.93 ^a (3.4)	8.25 ^a (0.9)	-0.55 ^a (0.1)	0.38 ^a (0.02)	25.28 ^a (3.6)	2.97 ^a (0.1)
24L	17.34 ^{ab} (2.9)	7.47 ^a (0.8)	-0.24 ^b (0.03)	0.47 ^a (0.04)	19.8 ^a (3.3)	2.61 ^a (0.3)
12L:12D	11.52 ^b (1.5)	5.72 ^a (1.0)	-0.43 ^{ab} (0.05)	0.48 ^a (0.05)	15.3 ^a (3.4)	2.52 ^a (0.2)

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447 **Table 5.** Mean values (\pm SEM) of the nutritional parameters from the 16L:8D group of the present study. Our results are
 448 compared with the results found in shi drum by Segato et al. (2006, 2007) (mean values \pm SD) and Zafer et al. (2012).

	16L:8D group (dorsal and ventral fillet)	Dorsal fillet (Segato et al., 2007)	Whole-body (Segato et al., 2006)	Whole -body (Zafer et al., 2012)
Moisture (%)	74.41 (0.44)	76.4 (0.2)	63.6 (0.5)	72
Protein (%)	21.24 (0.14)	21.5 (0.2)	18.9 (0.1)	18
Total fat (%)	2.66 (0.53)	0.5 (0.1)	13.5 (0.5)	4.3
Ash (%)	1.46 (0.06)	1.4 (0.1)	4.8 (0.1)	4.0

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453 **Fig. 1.** Transverse white muscle sections of 11.8 months old specimens (**a,b**) and commercial size specimens (**c,d**) from
454 the 24L (**a,d**), 16L:8D (**b**) and 12L:12D (**c**) groups. Bars **a**: 100 μm ; **b,d**: 66.66 μm ; **c**: 50 μm ; *W* white muscle fibres; *nW*
455 new white muscle fibres.

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