

METABOLIC AND REPRODUCTIVE RESPONSES IN NILE CATFISH "*CLARIAS GARIEPINUS*" EXPOSED TO DIFFERENT PHOTOPERIOD REGIMES

SAFAA, S. ABD EL-HAMID*, ABASS, H.I.**, and AHMED, H.H. **

*Biochemistry Department, Animal Health Research Institute, Dokki, Giza

** Physiology Department , Faculty of Veterinary Medicine, Cairo University.

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SUMMARY

After adaptation of sixty mature Nile Catfish *Clarias gariepinus* for 2 weeks under photoperiod regime (12L : 12D). Fish were allowed into six identical tanks. Each tank contains 10 fish (with equal numbers of male and female). Two tanks were remained on the conditioning photoperiod regime (12L : 12D) , other 2 tanks were changed to 24L: 00D and the last 2 tanks were subjected to 00L : 24D hours. All fish in the experimental tanks were remained for 5 consecutive weeks under the previously applied photoperiod regime. Continuous darkness period (00L : 24D) resulted in increased serum testosterone (T)level and gonadosomatic index (IG) compared to medium and extended periods of light (12L: 12D and 24L : 00D) in male *C. gariepinus* fish. Furthermore, no light (00L: 24D) and medium period of light regimes (12L:12D) showed higher serum estradiol

-17 β (E₂) levels and gonadosomatic index (IG) compared to respective values of continuous light regime (24 L: 00D) in female *C. gariepinus* fish. Fish under continuous light regime (24L: 00D) exhibited the highest values of serum cortisol , glucose, free fatty acids (FFA) and lactate levels compared to those who exposed to shorter period of light (00L : 24D). In conclusion, the results of this study revealed that the artificial photoperiod regime (light) plays an important role in Nile catfish , *Clarias gariepinus* reproduction. Fish were more stressed and aggressive when exposed to longer periods of light. Moreover, as the hours of light increased during the 24 h cycle, *Clarias gariepinus* fish exhibited a decrease in serum sexual steroid hormones (T and E₂) and gonadosomatic indices, which associated with an increase of serum

cortisol (F) concentrations in male and female *C. gariepinus* fish.

INTRODUCTION

African catfish, *Clarias gariepinus* are widely distributed throughout many African countries and also in North Israel as well as Nile river pathway and other freshwater resources in Africa. African catfish, *Clarias gariepinus* fish are important aquacultural species. African catfish is characterized by an annual spawning cycle that is highly dependent on water temperature, with spawning typically occurring during spring and early summer (El-Bolock, 1973; Van Oordt and Goos, 1987; Van Oordt et al., 1987; Van Den Hurk et al., 1986 and Van Weerd, et al, 1991).

African catfish, *Clarias gariepinus* like other *Clarias* species are carnivorous fish, so, the stocking density is considered the most important factor affecting cannibalism and aggression (Almazán Rueda, 2004). Reproductive success of this economically important species is highly variable.

Researches to improve reproductive success suggest that, like other captive silurids, e.g. *Clarias batrachus* (L) (Mannickam and Joy,1989); *Heteropneustes fossilis* (Bloch); (Tharakan and Joy, 1996) and *Ictalurus*

Key words: *Clarias gariepinus*; sexual steroid hormones; photoperiod; gonads; stress parameters

punctatus (Rafinesque) (Small, 2004 and Silverstein and Small, 2004), final gonadal maturation of *Clarias gariepinus* is often inhibited by factors other than stocking density such as photoperiod (Schreck et. al ,2001 and Almazán Rueda et al., 2004 and 2005).

Photoperiod requirements are species specific and vary for each developmental stage. Light and dark alteration is generally thought to be the main synchronizer of feeding activity (Hossain et al., 1999).only a few studies have assessed the effect of photoperiod on feeding activity and social behaviour and consequently alteration in hormone production which may improve feed conversion efficiency in *Clarias gariepinus* (Almazán Rueda, 2004 and Almazán Rueda et al., 2004 and 2005).

The aim of the present study was to clarify the influence of different photoperiods (12 L: 12 D, 24 L: 00 D and 00L: 24 D) on serum sex reproductive hormones (testosterone and estradiol,17- β) and stress variables (Cortisol, glucose, FFA and Lactates), of adult mature *C. gariepinus*. Furthermore, the effect of

these photoperiods on gonadal recrudescence asessed.

MATERIAL AND METHODS

Experimental set up:

Sixty adult mature African Nile catfish "*Clarias gariepinus*" (30 male and 30 female) with an average b.wt. 380 – 400 g of each sex. *C. gariepinus* were purchased alive in apparently good health from a fish market of Giza governorate during the non-breeding season (January- February, 2006). Fish were kept in 6 glass aquaria (100 X 50 X 60 Cm³) supplied with dechlorinated water (10 fish as 5 male and 5 female / Aquarium).

All fish were held in glass aquaria during an adaptation period, which lasted 2 weeks under a 12 L: 12 D photoperiod at 18 °C ± 2°C . Fish were fed during the experimental period with a formulated feed (commercial catfish diet 40% protein) in the form of floating (5 mm pellet size) by hand feeding regime once / daily (as long feeding behaviour was observed). Artificial light was designed at approximately 2 m distance from the water surface of the fish aquarium. The light source in this artificial illumination system was fluorescent lamps (Day light 40WATT: 120 cm length : FL 40T8D/ 36 FL 40T9D / 38, MFG Co. Ltd , Thiland) . Sufficient aeration

and gonadosomatic index (*I*G) were also

was supplied by air pumping compressor to the confinement aquarium to prevent external additional stress from oxygen depletion. Average aquarium water temperature was 18°C, range ± 2 °C.

All fish in the experimental tanks were subjected to 12L: 12D, 24L : 00D and 00L: 24D / regime respectively for 5 consecutive weeks (Biswas and Takeuchi, 2003).

For the photoperiod of 00L : 24D, lights were off all the time, for the period 24L : 00D, light were on all the time and for 12L :12D, lights were on from 0700 to 1900 hours, respectively (Almazán Rueda et al., 2004).

Blood sampling technique:

At the end of experimental period, fish were anaesthetized with tricaine methanesulfonate (MS-222, 0.35 g l⁻¹) (Crescent research chemicals, Phoenix, AZ, USA) and 0.7 g l⁻¹ of NaHCO₃) at 07.00 AM , to minimize stress throughout sampling period . Blood was collected by hypodermic syringe from the caudal vessels, within 2 min. after being anaesthetized. The collected blood sample of each fish was left to coagulate at room temperature. Serum was separated by

centrifugation at 3000 rpm for 5 min. The collected sera were stored at -30 C° for subsequent analysis of sera levels of cortisol, glucose, FFA, lactates, testosterone and estradiol-17β.

Gonadosomatic Index (IG):

Live body weight of each fish was determined before slaughtering. The gonadosomatic index (IG) was computed as % weight of gonads of each sex to the gutted weight of *C. gariepinus* fish.

$IG = 100 (\text{Gonads Wt.}) (\text{Gutted fish Wt.})^{-1}$, according to Silverstein and Small (2004).

Hormonal and chemical assays:

1. Serum cortisol (F) was determined using cortisol "RIA-DSL 2100, USA" Kit^{*} according to the method adopted by Schlaghecke et al., (1992).

2. Serum testosterone (T) was determined using testosterone "RIA-DSL4000, USA" Kit^{*} according to the method of Jaffe and Behrman (1974).

3. Serum estradiol -17-β (E2) was determined using estradiol -17-β "RIA-DSL43100, USA" Kit^{*} according to the method of Xing et al. (1983) and Hammond (1990).

* Diagnostic Systems Laboratories, Inc. Webster, Texas, USA (DSL).

4. Serum glucose was determined spectrophotometrically using enzymatic Kit according to the method adopted by Trinder (1969).

5. Serum lactate was determined enzymatically using Sigma diagnostic Kit (Sigma USA) according to Noll (1974).

6. Serum free fatty acids (FFA) was determined spectrophotometrically using chemical method after Larsson and Fange (1977).

Statistical analysis:

All data were subjected to statistical analysis of variance (ANOVA) according to the procedures reported by Snedecor and Cochran (1980) and SAS (1990). LSD was calculated and taken in consideration only, where F value was significant.

RESULTS AND DISCUSSION

Data presented in tables (1&2) and figures (1&2) revealed that Nile African catfish, *Clarias gariepinus* exposed to continuous period of light (24L: 00D) exhibited the maximal values of stress variables (cortisol, glucose, free fatty acids "FFA", and lactate) in both sexes of mature fish as compared to those who subjected to shorter periods of light

(12L:12D and 00L:24D). The results of the present investigation showed a positive direct correlation between extended light photoperiod (24L:00D) and serum values of cortisol hormone "the hormone of stress" and consequently glucose and other stress metabolites such as FFA and lactate.

The present results recorded in tables (1&2) are in agreement with those of (Almazán Rueda et. al., 2004 and 2005) who found that African catfish, *Clarias gariepinus* juveniles reared under a longer light photoperiod (06D:18L) showed the highest levels of plasma cortisol, free fatty acids and lactate, compared to those who were reared at shorter period of light (18D:06L and 24D:00L) respectively. Data recorded in tables (1&2) and figures (1&2) revealed an increase of serum cortisol levels in both sexes of fish exposed to continuous light regime (24L:00D). These data were compared to respective values of those who were subjected to shorter light photoperiods (12L:12D and 00L:24D). The increase of cortisol can be due to the stress of high swimming activity, searching for cover and aggregation. A secondary response of the high levels of cortisol is the increased in serum glucose and FFA. This increase in serum glucose levels may be due to the glycolytic effect of cortisol (Trenzado et.al., 2003). The increase in serum FFA levels may be in part due to elevated concentration of cortisol in the fish (Almazán Rueda et. al., 2004 and 2005

and Ruane et. al., 2001 and 2002) as lipolysis is stimulated by cortisol and catecholamines.

Almazán Rueda (2004) and Almazán Rueda et.al. (2004 and 2005) in African catfish, *C.gariepinus* and Biswas et.al. (2004) in Nile tilapia, *Oreochromis niloticus* showed that as the hours of light increased during the 24 h. cycle, fish became more stressed and aggressive, compared to those under a reduced number of light hours. *Clarias gariepinus* juveniles reared under longer period of light (12L:12D and 18L:6D) showed higher swimming activity, more aggression and higher plasma cortisol, glucose, total non-estrified fatty acids (FFA) levels compared to those who reared at shorter periods of light (06L: 18D and 00L:24D) (Almazán Rueda et.al. , 2005).

Concerning the role of photoperiod manipulation on fish production; data presented in this study (Tables 1&2 and Figures 1&2) displayed that sexual steroid hormones, testosterone (T) in male and 17 β -estradiol (E2) in female Nile catfish, *C. gariepinus* reached their maximal values in fish exposed to continuous darkness (00L: 24D) as compared to respective values of those who were subjected to longer periods of light (12L:12D and 24L: 00D) respectively. Moreover, gonadosomatic indices (*I*G) of

both sexes of *C. gariepinus* fish revealed that fish exposed to continuous light regime (24L:00D) exhibited minimal values compared to those who were exposed to (00L:24L) in male fish and to (12L:12D and 00L: 24D) in female fish, respectively. As seen presently, it is observed that there is a negative effect of physiological stress (cortisol elevation) on reproductive sexual steroid levels via photoperiod manipulation in Nile catfish (Tables 1&2).

Our results are in agreement with many researchers who demonstrated that stress has the capacity to inhibit reproductive performance in fish (Small, 2004 and Silverstein and Small, 2004), as exhibited by stress in tilapia , *Oreochromis niloticus* (Biswas et.al., 2004) and channel catfish , *Ictalurus punctatus* (Small,2004 and Schreck et.al., 2001).

However, the association between stress, elevations in plasma cortisol (F) and concomitant depressions in plasma testosterone (T) and 17 β - estradiol (E₂) levels in Snapper, *Pagrus auratus* (Carragher & Pankhurst, 1991) together with evidence that exogenous cortisol (F) produces inhibitory effects on reproduction in Salmonids (Pottinger et.al., 1991), in roach fish (Pottinger et.al.,1999), tilapia *Oreochromis niloticus* (Foo and Lam, 1993), Snapper (Cleary et. al., 2000), spiny damselfish, *Acanthochantrichromis polyacanthus* (Pankhurst , 2001), has lead to the general assumption that the effect of stress on fish reproduction is mediated through the action of cortis

l (F) on the gonads. Contrary to our findings, Pankhurst and Van Der Kraak (2000) demonstrated no effect of elevated plasma cortisol levels on E₂ or maturational gonadotropin (GtH) in rainbow trout, but found plasma T levels to decline in a stepwise manner over time. However, Pankhurst and Van Der Kraak (2000) concluded that the potential inhibitory effects of cortisol on male fish reproduction do not involve of gonadotropin (GtH) secretion, and may possibly be at the level of gonadotropin (GtH) signal transduction. Additionally, Small (2004) and Silverstein & Small (2004) demonstrated that despite the negative previous results of cortisol on fish reproduction, cortisol-fed channel catfish had an average of 47.1% more spwans than the control-fed fish, with no observable effects on the egg quantity or quality as determined by hatching success, concluded that the cortisol does not suppress channel catfish reproduction.

Conclusively, the results of this study revealed that the artificial photoperiod regime (light) plays an important role in Nile catfish (*Clarias gariepinus*) reproduction. Fish were more stressed and aggressive when exposed to longer periods of light. Moreover, as the hours of light increased during the 24 h cycle, the fish exhibited a decrease in serum sexual steroid hormones (T and E₂) and gonadosomatic indices, which associated with an increase of serum cortisol (F) concentrations.

Table (1): Serum testosterone , cortisol, glucose, free fatty acids (FFA), lactate, and gonadosomatic index (I_G) of male Nile catfish *Clarias gariepinus* exposed to different photoperiod regimes

Parameters Light :Dark Photoperiod	Testosterone (ng dl ⁻¹)	Cortisol (ng ml ⁻¹)	Glucose (mg dl ⁻¹)	FFA (mg dl ⁻¹)	Lactate (mg dl ⁻¹)	I_G
12L:12D	232.70 ± 7.78 ^a	147.30 ± 5.13 ^a	50.90 ± 3.12 ^a	42.1 ± 3.68 ^a	35.00 ± 3.60 ^{ab}	0.343 ± 0.033 ^a
24L : 00 D	160.70 ± 8.08 ^b	188.30 ± 5.41 ^b	75.00 ± 3.55 ^b	54.8 ± 3.75 ^b	43.30 ± 3.16 ^a	0.307 ± 0.039 ^a
00 L: 24 D	390.30 ± 7.77 ^c	132.40 ± 4.69 ^c	39.10 ± 3.44 ^c	39.2 ± 3.51 ^a	29.70 ± 2.72 ^b	0.410 ± 0.048 ^b
F	1240.95	32.40	29.39	5.17	4.64	5.505
LSD	9.66	14.74	9.79	10.58	9.23	0.065

Mean values ± SE

ANOVA single way , n=10.

Mean values having the same letter(s) in the same column are non-significantly different from each other at P<0.05

$I_G=100(\text{Testis mass}) (\text{Body mass})^{-1}$

Table (2) : Serum estradiol-17 β , cortisol, glucose, free fatty acids (FFA), lactate, and gonadosomatic index (I_G) of female Nile catfish *Clarias gariepinus* exposed to different photoperiod regimes

Parameters Light :Dark Photoperiod	Estradiol-17 β (pg ml ⁻¹)	Cortisol (ng ml ⁻¹)	Glucose (mg dl ⁻¹)	FFA (mg dl ⁻¹)	Lactate (mg dl ⁻¹)	I_G
12L:12D	143.60 \pm 5.04 ^a	135.20 \pm 3.16 ^a	45.7 \pm 0.367 ^a	37.6 \pm 0.54 ^a	27.7 \pm 0.93 ^a	2.10 \pm 0.045 ^a
24L : 00 D	97.40 \pm 4.35 ^b	164.80 \pm 6.99 ^b	60.7 \pm 0.616 ^b	48.3 \pm 0.79 ^b	35.6 \pm 0.56 ^b	1.79 \pm 0.031 ^b
00 L: 24 D	212.40 \pm 4.25 ^c	117.90 \pm 4.87 ^c	34.8 \pm 0.467 ^c	31.8 \pm 0.47 ^c	25.2 \pm 0.33 ^c	2.56 \pm 0.031 ^c
F	160.91	751.498	693.89	185.28	68.53	114.81
LSD	13.23	2.51	1.431	1.78	1.90	0.104

Mean values \pm SE

ANOVA single way, n=10.

Mean values having the same letter(s) in the same column are non-significantly different from each other at P<0.05

$I_G = 100(\text{Ovary mass}) / (\text{Body mass}) - 1$

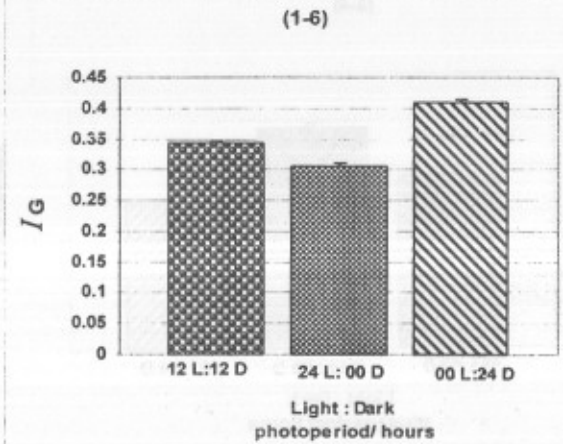
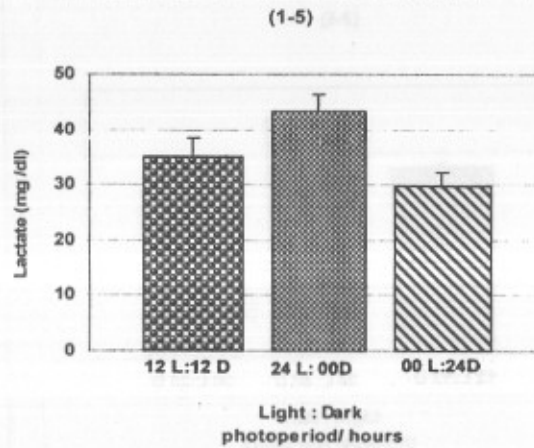
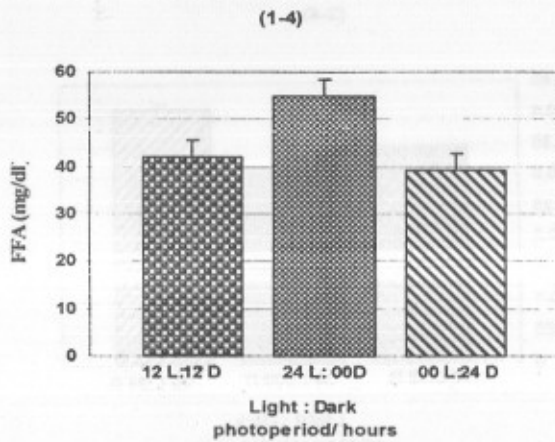
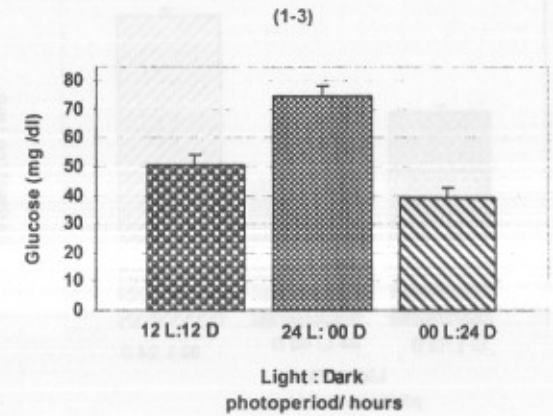
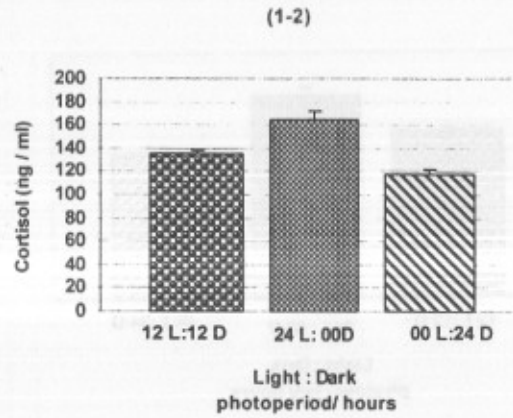
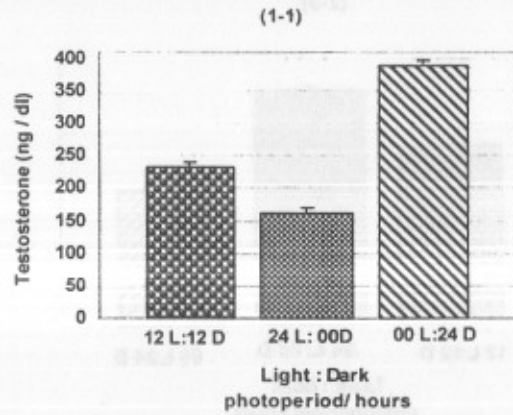


Fig.(1) : Serum testosterone, cortisol, glucose, free fatty acids (FFA), Lactate, and gonadosomatic index (IG) of male Nile catfish *Clarias gariepinus* exposed to different photoperiod regime

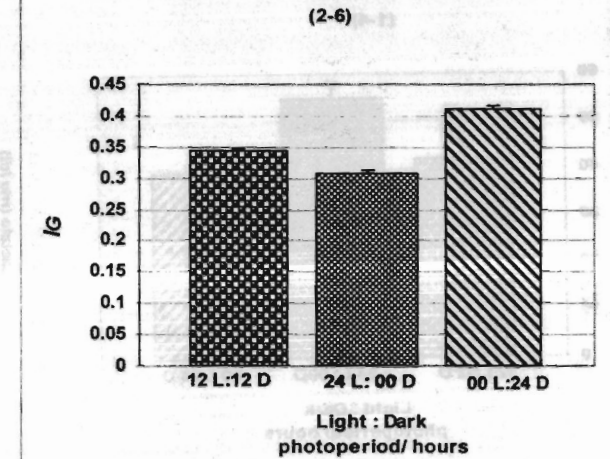
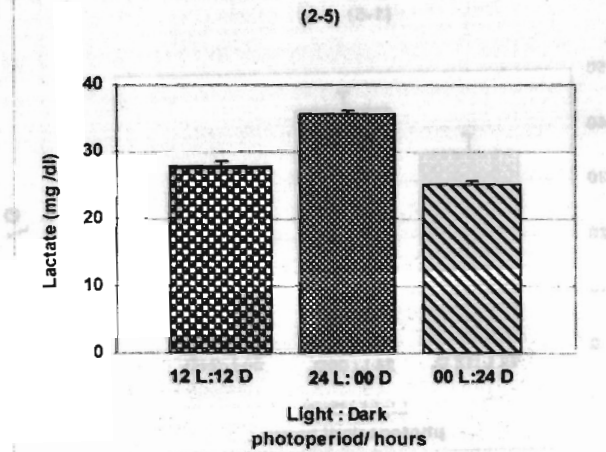
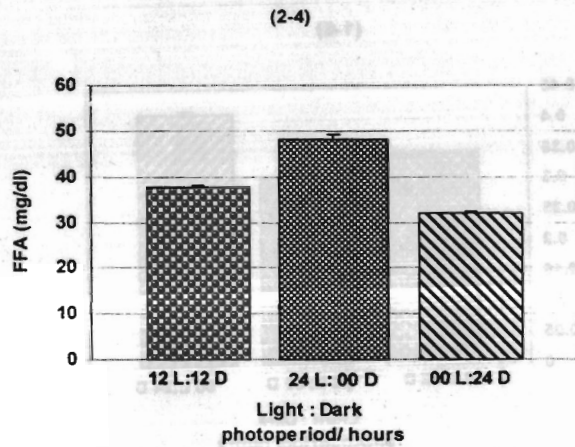
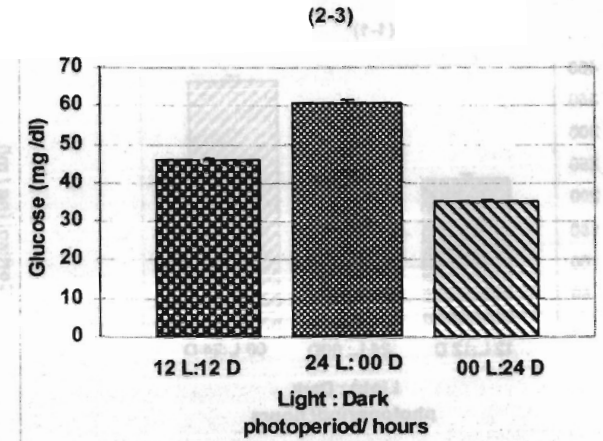
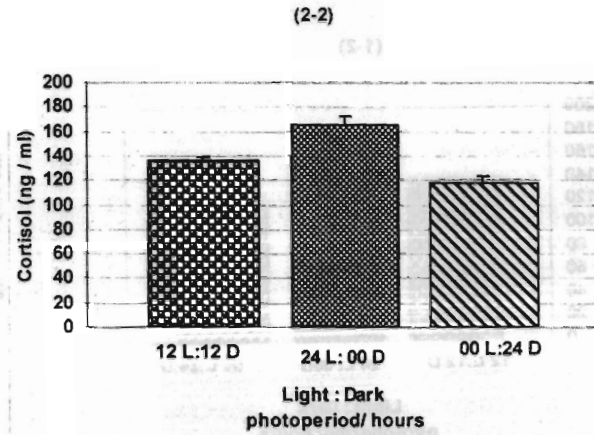
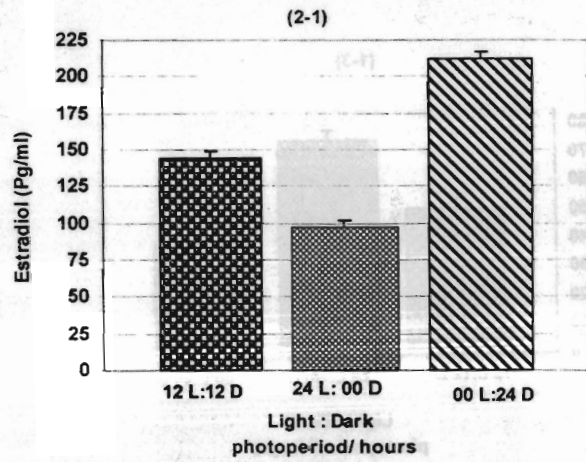


Fig.(2) : Serum Estradiol-17 β , cortisol, glucose, free fatty acids (FFA), Lactate, and gonadosomatic index (IG) of female Nile catfish *Clarias gariepinus* exposed to different photoperiod regimes

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الاستجابات الأيضية والتناسلية للقراميط النيلية المعرضة لأنظمة مختلفة من الإضاءة

* صفاء سيد عبد الحميد - ** حسن إبراهيم عباس - ** هدى الله حاتم أحمد
* قسم الكيمياء الحيوية والنقص الغذائي والسموم بمعهد بحوث صحة الحيوان - الدقي - الجيزة
** قسم الفسيولوجيا - كلية الطب البيطري - جامعة القاهرة

بعد فترة الأكلمة والتي استمرت لمدة أسبوعين متتاليين لعدد 60 قرموط نيلي ناضج (كلاريس جاريبينس) تحت نظام إضاءة (12 ساعة ضوء : 12 ساعة إظلام). وزعت مجموعة القراميط جميعها في 6 أحواض متماثلة بحيث يحتوي كل حوض زجاجي على 10 أسماك (5 ذكور و 5 إناث). وبعد ذلك تم الاستمرار في تعريض حوضين منهم إلى نفس النظام الإضاءة السابق وحوضين آخرين لنظام إضاءة (24 ساعة ضوء : صفر ساعة إظلام) أما الحوضين الآخرين فتم تعريضهما لنظام إضاءة (صفر ساعة ضوء : 24 ساعة إظلام). واستمر تعريض جميع أسماك القراميط لنظم الإضاءة المختلفة السابقة لفترة 5 أسابيع متتالية.

وقد أوضحت الدراسة أن مجموعة الأسماك المعرضة للإظلام التام (صفر ساعة ضوء : 24 ساعة إظلام) كان مستوى هرمون التيستوستيرون في أمصال ذكور القراميط الجاريبينس وكذا معامل الجنس : جسمي في أعلى مستوى له عن المجموعتين الأخرين المعرضتين لفترات إضاءة أطول (12 ساعة ضوء : 12 ساعة إظلام) و (24 ساعة ضوء : صفر ساعة إظلام). علاوة على ذلك فقد أوضحت نتائج الدراسة أن نظام عدم الإضاءة الكاملة (صفر ساعة ضوء : 24 ساعة إظلام) و نظام عدد متوسط الإضاءة (12 ساعة ضوء : 12 ساعة إظلام) قد أدى إلى ارتفاع مستوى هرمون الإستراديول - 17 ب في أمصال إناث القراميط وكذا مؤشر الجنس : جسمي فيها إلى قيم أعلى منه عن مثيلاتها في المجموعة المعرضة لنظام الإضاءة المستمرة (24 ساعة إضاءة : صفر ساعة إظلام).

وقد أثبتت الدراسة أن تعرض القراميط للإضاءة المستمرة (24 ساعة ضوء : صفر ساعة إظلام) قد أدى إلى زيادة قصوى في قيم هرمون الكورتيزول وكذا الجلوكوز والأحماض الدهنية الحرة واللاكتات في أمصال ذكور وإناث القراميط عنها في المجموعتين الأخرين المعرضتين لساعات إضاءة أقل (12 ساعة ضوء : 12 ساعة إظلام) و (صفر ساعة ضوء : 24 ساعة إظلام).

ومما سبق يتضح أن الضوء (النظام الاصطناعي للإضاءة) يلعب دوراً هاماً وحيوياً في عملية التناسل في القراميط النيلية (كلاريس جاريبينس) وأنه كلما زادت ساعات الإضاءة خلال الـ 24 ساعة/ يوم كلما ثبت ذلك من مستوى هرمون التيستوستيرون في الذكور وهرمون الإستراديول في الإناث مع خفض في مؤشر الجنس : جسمي وكان ذلك مصحوباً بارتفاع واضح في مستوى هرمون الكورتيزول في أمصال تلك الأسماك من الجنسين.