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PHOTOPERIOD AND SALINITY EFFECTS ON SURVIVAL AND SOME BLOOD PARAMETERS IN FEMALE *BUFO REGULARIS*

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ABSTRACT

This study, investigates the effect of different salinities injected (30, 70 and 100 % sea water) and photoperiods (L : D / 8 : 16, L : D / 12 : 12 and L : D / 16 : 8) on female *Bufo regularis* (1) survival rate, (2) oxygen and carbon dioxide partial pressure, and (3) sodium and potassium contents. Undiluted sea water lowered the survival rate. while the highest survival rate occured at L : D / 16 : 8 combined with 30 % SW. PO_2 was lower in all experimental groups than in the controls. PO_2 tension was higher in the blood of females exposed to L : D / 16 : 8 in all groups, except for those injected with 30 % sea water, than that in those subjected to L : D / 8 : 16 or L : D / 12 : 12. In all groups, Na and K concentration inereased with the increase in sea water concentration. Ion concentration in females injected with 70 % or 100 % SW was Lower in the plasma of frogs exposed to L : D / 12 : 12 than that in those exposed to L : D / 8 : 16 or L : D / 16 : 8.

INTRODUCTION

Some amphibians are permanently aquatic; others spend the minority of their lives on land. A few frogs are known to breed in brackish water, but none are truly marine (Prosser, 1973).

In modern Amphibia, the skin is the prime area for oxygen uptake at low temperature (Less than 10°C) and the lung predominates at higher temperature (more than 10 - 13°C) while the skin is always the major area of carbon dioxide elimination (Guimond and Hutchinson, 1968). The latter authors found that

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Ambystoma opacum takes in 34 % of its oxygen requirement by lungs and 66 % by skin, 80 % of carbon dioxide was eliminated through the skin (Cloudsley–Thompson, J.L., 1970).

Dehnel (1958) found that oxygen consumption of fresh water crabs tends to be higher than that of marine ones. He also reported that when individuals were transferred from sea water to reduced salinity, oxygen consumption was enhanced.

Rao (1958) compared the oxygen consumption of the prawn *Metopenaeus monoceres,* from marine and brackish water habitats. The brackish water prawn had minimum oxygen consumption in 50 % sea water which was equivalent to their natural habitat and probably closest to the osmotic content of their body fluids, metabolic rates were elevated in both lower and higher salinities. In contrast, prawns from marine habitats had minimal rates in 100 % sea water which is their natural habitat and is isotonic with their body fluids.

Newell (1970) summarized some of the important experiments on the intertidal invertebrates. The most consistent pattern was minimum metabolic rates at salinities which were closest to the osmotic content of the body fluids. It seemed that the metabolic costs of ion – osmoregulation were major factors in determining the effects of salinity on oxygen consumption.

Experimentally, the shapes of oxygen equilibrium curves can be readily changed by varying the salt concentration of the medium. Increasing the Na Cl concentrations, for instance, can promote the dissociation of oxyhaemoglobin (Riggs, 1965).

Dehnel (1958) found that photoperiod can affect the metabolism in the crustacean *Pachgrapsus.* On an 8 hour photoperiod this anthropod consumed more oxygen than when it was on a 16 hour one. Circadian and seasonal rhythms in oxygen consumption are often well marked. They are usually correlated with the animal's normal activity rhythm. Experimentally, photoperiod has been shown to modify oxygen consumption in certain animals, it can be assumed that light triggers these changes through neurosecreters and changing hormone levels (Hoar, 1975).

It is of interest that the pH of the poikilotherms at 37°C is very colse to that of the homeotherms and this may suggest that the blood pH of the hibernator changes when the body temperature is depressed (Hoar, 1975).

Fry (1971) reported that Na Cl injection caused plasma crystallization, oxygen pressure rise, water movement from the interstitial spaces into the plasma with

simultaneous salt diffusion out into the intertitial spaces.

The present study investigates the effect of different salinities and photoperiods on (1) O_2 and CO_2 tensions of fully oxygenated blood, (2) plasma Na and K concentrations and (3) survival rate of female Bufo regularis (Cloudsley-Thompson, J. L., 1967).

MATERIALS AND METHODS

A colony of *Bufo regularis* originating from Cairo, Egypt was bred in Qatar Agricultural Station from 1976. Female *Bufo regularis* were collected during April and May and were allowed to acclimate in the laboratory (ventilated, sunny and at 25 + 2 °C) for one week before experimentation.

Photoperiod: 3 different photoperiods, L : D / 8 : 16, L : D / 12 : 12 and L : D / 16 : 8, were tested. A 20 W fluorescent Light bulb was used in a photoperiod cabinet (60 × 90 × 35 cm).

Salinity: Four groups of animals were held under each photoperiod. Each of 3 groups was daily injected subcutaneoualy with 0.1 ml of 30 % sea water (SW), 70 % SW or 100 % SW, and fourth group was used as a control. Six to 10 females ware used in each group and each experiment was replicated 3 times. They were kept in plastic aquaria (14 × 21 × 15 cm) with a little moist grass and fed on ground beef.

Survival rate: The number of surviving females was determined daily for all groups until all animals died.

Blood gases and concentration: The above experiments were repeated and in all groups the frogs were daily injected (except the control ones) for 4 – 5 days with the different SW concentrations. Blood was collected by cutting blood vessels in the neck region and collecting the blood in a heparinized petridish (1.000 USP units / ml of blood) Blood gases were measured by fully oxygenating the sample with air at 25° for 30 minutes equilibrium period. This period was enough to saturate the blood with oxygen when exposed to atmospheric air (Wood and Lenfort, 1975). The sample was then analyzed for gas contents (as pertial pressure (mm Hg) of oxygen (PO_2), and of carbon dioxide (PCO_2). Corning 168 PH Blood Gas Analyzer (Essex, England Co. 92 DX) was used to measure PO_2 and PCO_2.

Blood samples collected as described above were centrifuged and a Corning 400

flame photometer was used to measure Na and K concentrations (P P m) in the separated plasma of all groups.

The means and standard errors were calculated for all groups. The data were compared using the student's t – test and analysis of variance (F – ratio).

RESULTS

1. Survival Rate

A. Effect of Salinity

At L : D / 8 : 16 in all experimental groups and control, 50 % of the females died in 4 days or less and all females died within 5 days (Fig. 1 A). At L : D / 12 : 12 and L : D / 16 : 8, 50 % and 100 % mortality in control females and those injected with 100 % SW were similar to that in L : D / 8 : 16. In those injected with 70 % SW, 50 % mortality occurred after about 6 days and all died within 7 days, in frogs injected with 30 % SW, 50 % mortality in L : D / 12 : 12 occurred in less than 6 days and in L : D / 16 : 8 in 8 days : all females died within 10 and 9 days respectively (Fig. 1B, C).

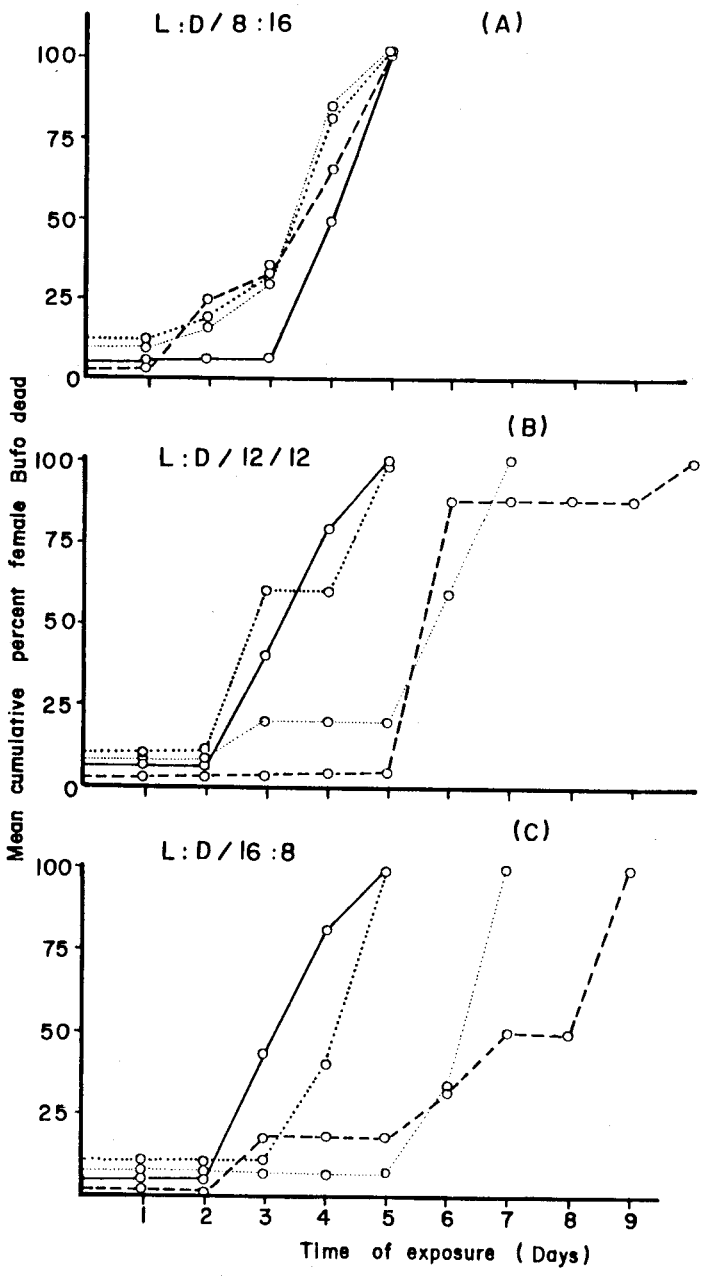
B. Effect of Photoperiod

Females treated with 30 % or 70 % SW, survived longer at L : D / 16 : 8 and L : D / 12 : 12 than at L : D / 8 : 16 while control females and those injected with 100 % SW survived for similar periods under all photoperiod conditions. (Fig. 1 A, B, C).

2. Blood Gas Contents

A. Effect of Salinity

Salinity did not affect the PO_2 in frogs exposed to L : D / 8 : 16 or L : D / 12 : 12 (Table 1). However, in females subjected to L : D / 16 : 8 PO_2 was lower (P less than 0.05) in the group injected with 30 % than in those injected with 70 % or 100 % SW (Table 1). PO_2 in all experimental groups was lower (P less than 0.01) than that of the controls.



Effects of different concentration of sea water (o - - - o 30 %, o ..... o 70 %, o ..... o 100 % SW and o—o control) on survival of female *Bufo regularis* exposed to three different photoperiods : L : D / 8 : 16, L : D / 16 : 8, and L : D / 12 : 12.

At L : D / 8 : 16 PCO_2 was higher (P less than 0.05) in the group injected with 100 % SW than in the control group and in those injected with 30 % SW (Table 1). No significant difference (P more than 0.05) occurred between the PCO_2 of animals injected with 70 % SW and those injected with 100 % SW. For females exposed to L : D / 12 : 12 or L : D / 16 : 8 PCO_2 was higher (P less than 0.05) in the groups injected with 70 % and 100 % SW than those injected with 30 % SW (Table 1); PCO_2 in the latter group was higher than that in the control group (P less than 0.01).

B. Effect of Photoperiod

PO_2 was higher (P less than 0.01) in the blood of females exposed to L : D / 16 : 8 in all groups, except for those injected with 30 % SW, than that of females exposed to L : D / 8 : 16 or L : D / 12 :12 PO_2 of the corresponding groups maintained in the latter 2 photoperiods was nearly similar (P more than 0.05) (Table 1).

On the other hand, PCO was higher (P less than 0.01) in the blood of control froges subjected to L : D / 8 : 16 or injected with 30 % SW than the corresponding groups held in L : D / 12 : 12 or L : D / 16 : 8. Photoperiod did not alter (P more than 0.05) PCO_2 in females injected with 70 % or 100 % SW (Table 1).

3. Plesma Ion Concentrations

A. Effect of salinity

Na and K concentrations were higher (P less than 0.01) in plasma of all females injected with sea water than that in the controls (Table 2), except ion contents of frogs injected with 30 % SW in L : D / 12 : 12 or L : D / 16 : 8. However, in all groups ion concentration increased (P less than 0.01) with increase in SW concentration.

Table 1

Mean partial pressures of oxygen (PO_2) and carbon dioxide (PO_2) of fully oxygenated blood of female frogs *Bufe regularis* injected subcutaneously for consecutive 4 – 5 days with different salinities (30 %, 70 % and 100 % sea water (SW) and exposed to photoperiods of L : D / 8 : 16, L : D / 12 : 12, L : D / 16 : 8

Salinity Groups	Mean ± SE					
	L : D / 8 : 16		L : D / 12 : 12		L : D / 16 : 8	
	PO_2	PCO_2	PO_2	PCO_2	PO_2	PCO_2
Control	$86.5 \pm 0.46^{c*}$	25.2 ± 2.31^{a}	$93.7 \pm 1.42^{d,e}$	9.4 ± 0.56^{i}	148.9 ± 0.62^{h}	12.8 ± 3.34^{i}
30 % SW	81.8 ± 1.73^{c}	26.1 ± 2.83^{a}	73.9 ± 1.23^{e}	10.8 ± 0.29^{i}	68.7 ± 0.64^{g}	20.8 ± 0.87^{a}
70 % SW	$86.1 \pm 8.99^{c,d}$	$31.2 \pm 7.37^{a,b}$	$77.1 \pm 5.49^{c,e}$	25.5 ± 1.44^{a}	100.5 ± 3.19^{f}	$32.5 \pm 5.46^{a,b}$
100 % SW	87.5 ± 0.84^{c}	36.9 ± 3.58^{b}	$86.8 \pm 5.32^{c,d,e}$	$30.7 \pm 0.64^{a,b}$	105.3 ± 1.37^{f}	34.4 ± 6.96^{b}

* Figures with similar letters are not significantly different (P more than 0.05), those with different letters are statistically different (P less than 0.01).

B. Effect of photoperiod

In L : D / 16 : 8 ion concentration in plasma of females injected with 30 % SW were lower (P less than 0.01) than that of those exposed to L : D / 8 : 16 or L : D / 12 : 12. In L : D / 12 : 12 ion concentration in females injected with 70 % SW was lower (P less than 0.01) than that of those exposed to L : D / 8 : 16 or L : D / 16 : 8. In females injected with 100 % SW Na concentration was nearly similar in females of the 3 photoeriods while potassium concentration was lower in plasma of females exposed to L : D / 12 : 12 than that of those exposed to L : D / 8 : 16 or L : D / 16 : 8 (Table 2).

Table 2

Mean concentration of Na^+ and K^+ (p p m) in plasma of female Bufo regularis injected subcutaneously for consecutive 4 – 5 days with different salinities (30 %, 70 % and 100 %) sea water (SW) and exposed to photoperiode of L : D / 8 : 16, L : D / 12 : 12, L : D / 16 : 8

Salinity Groups	Mean ± SE					
	Na			K		
	L : D / 8 : 16	L : D / 12 : 12	L : D / 16 : 8	L : D / 8 : 16	L : D / 12 : 12	L : D / 16 : 8
Control	$0.57 \pm 0.02^{a*}$	$2.55 \pm 0.02^{c,f}$	1.23 ± 0.019^{g}	1.05 ± 0.01^{b}	4.85 ± 0.07^{s}	2.38 ± 0.01^{q}
30 % SW	1.03 ± 0.01^{b}	1.08 ± 0.01^{b}	0.78 ± 0.01^{e}	2.48 ± 0.01^{k}	1.58 ± 0.01^{h}	1.23 ± 0.02^{g}
70 % SW	2.55 ± 0.02^{c}	1.28 ± 0.01^{g}	2.45 ± 0.02^{i}	6.25 ± 0.02^{n}	2.98 ± 0.01^{f}	3.85 ± 0.02^{m}
100 % SW	2.65 ± 0.02^{d}	$2.55 \pm 0.07^{d,f,l}$	2.75 ± 0.02^{l}	7.45 ± 0.02^{r}	5.05 ± 0.02^{s}	5.25 ± 0.02^{t}

* Figures with similar letters are not significantly different (p more than 0.05) : those with different letters are statistically different (p less than 0.01).

Analysis of the data showed that salinity and photoperiod significantly affect the oxygen content of female *Bufo* blood. Also, the analysis indicated that there was a significant correlation between Na and K ions and each of the salinity and photoperiod factors (Table 3).

Table 3

Analysis of variance (F–ratio) of partial pressures of oxygen (PO_2) and carbon dioxide (PO_2) and of sodium (Na), potassium (K) concentration in blood of female frogs *Bufo regularis* injected subcutaneoualy 4 – 5 days with different salinities (30 %, 70 % and 100 %) sea water (SW) and exposed to different photoperiods L : D : 16, L : D / 12 : 12, L : D / 16 : 8

Source of variation	F – ratio			
	PO_2	PCO_2	Na	K
Salinity	* 32.33	0.08	* 40.00	* 12.05
Photoperiod	* 33.15	0.04	* 18.80	* 15.94
Salinity and photoperiod	* 14.36	0.01	* 12.02	* 29.36

* Highly significant (P less than 0.001).

DISCUSSION

The results of this investigation show that survival rate of *Bufo regularis* at longer photoperiods is affected by the concentration of injected SW, i.e. the highest SW caused low survival rate. On the other hand, the longer light period caused high survival rate at the lower saline concentration. These results are in agreement with those of wilson (1979) who reported that survival rate in a given animal differed with the time of exposure to light.

In the present work, oxygen content in female *Bufo regularis* subjected to L : D / 16 : 8 was lower in the group injected with 30 % than in those injected 70 % or 100 % sea water. These findings agree with those of Dehnel (1958) who reported that when crabs transferred from sea water to reduced salinity. oxygen content decreased. Moreover, the data confirm the results of Riggs (1965) who found that increasing salt concentration can promote dissociation of oxyhaemoglobin.

In this work, oxygen content was higher in blood of *Bufo* subjected to L : D / 16 : 8 in all groups, with the exception of the 30 % SW group, than that of those exposed to L : D / 8 : 16 or L : D / 12 : 12. These findings confirm those of Dehnel (1958) and Hoar (1975) who concluded that photoperiod modifies oxygen consumption by altering the medium in which the enzymatic process of metabolism operate. Environmental factors (such as photoperiod and salinity) which operate in this way are controlling factors (Fry, 1971) : they govern both the maximum and minimum rates of metabolism while the limiting factor acts only on the active metablism.

In the present work, Na and K concentration, after injection with 70 % and 100 % SW was lower in the plasma of females exposed to L : D / 12 : 12 than that of those exposed toL : D / 8 : 16 or L : D / 16 : 8. Moreover, ion concentration was lower in the plasma of femaies injected with 30 % SW than that of those injected with 70 % or 100 % SW. These results agree with those of Fry (1971) who found that salt concentration increased in the plasma of fish with the increase in saline concentration.

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تأثير فترات إضاءة وتركيزات ملحية على بقاء وبعض معايير الدم لأنثى الضفدعة بوفورجيو لارس

أحمد الوزير هجرس

لقد أجريب الدراسة على أنثى الضفدعة **بوفورجيولارس** لمعرفة تأثير تركيزات ملحية مختلفة (٣٠ و ٧٠ و ١٠٠ ٪ ماء بحر) وفترات اضاءة مختلفة (٨ و ١٢ و ١٦ ساعة في اليوم) على : (١) معدل البقاء (٢) والضغط الجزئي لكل من الأكسجين وثاني أكسيد الكربون (٣) وتركيز البوتاسيوم والصوديوم ، وقد أثبتت النتائج أن ماء البحر غير المخفف يخفض معدل البقاء بينما لوحظ أعلى معدل للبقاء في الاناث التي تم حقنها بتركيز ٣٠ ٪ من ماء البحر اضاءة ١٦ ساعة في اليوم وقد انخفض الضغط الجزئي للأكسجين في جميع المجموعات اللاتي تم حقنها عن الضغط الجزئي في المجموعة غير المحقونة . كما لوحظ ارتفاع الضغط الجزئي للأكسجين في الاناث المعرضة لاضاءة ١٦ ساعة في اليوم عن مثيله في الاناث المعرضة لاضاءة ٨ أو ١٢ ساعة في كل المجموعات عدا تلك التي تم حقنها بتركيز ٣٠ ٪ من ماء البحر ، وقد ارتفع تركيز الصوديوم والبوتاسيوم في بلازما كل المجموعات بارتفاع التركيز في ماء البحر المحقون بينما انخفض تركيز الأيونات في الاناث التي تم حقنها بالتركيزات ٧٠ أو ١٠٠ ٪ من ماء البحر تحت اضاءة ١٢ ساعة في اليوم عن التركيز الأيوني في الأنثى المعرضة للضوء لمدة ٨ أو ١٦ ساعة .