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MONITORING THE STRESS ROLE IN THE PROMOTION OF YERSINIOSIS AMONG CULTURED OREOCHROMIS NILOTICUS (O. NILOTICUS) IN EGYPT

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ABSTRACT

The effect of several stressors in promotion of yersiniosis among farmed *O. niloticus* was evaluated. *Yersinia* infected fish were stressed by low dissolved oxygen (DO), increased unionized ammonia (NH3) and different copper levels (1mg/L for 1 hr, 0.15 mg/L for 10 days and 1mg/L for 1 month) at two different temperatures (18 ° C and 25°C). Decreased DO and exposure to copper increased mortality and/ or morbidity percentages. yersiniosis affected fish showed acute & subacute forms with establishment of asymptomatic carriers. Skin darkening with or without other septicaemic lesions was the principal clinical abnormalities. High percent of asymptomatic survivors were noticed among excess NH3 exposed fish. After 60 days post infection, all or most of unstressed and stressed survivors were *Yersinia* carriers. Significant reduction of Hb and total serum protein were observed among unstressed infected fish. All stressors caused acute anaemia with non significant decrease of total protein.

INTRODUCTION

Yersiniosis is an acute to chronic septicaemic stress related disease, now, generally recognized to be world wide (**Rintamaki *et al.*, 1986**). In Egypt, *Yersinia ruckeri* (*Y. ruckeri*), the causative agent of yersiniosis – was recovered from apparently healthy feral *O.niloticus* , *Clarias lazera* (*C. lazera*) , *Cyprinus carpio* (*C.carpio*) and *Chrysichthys auratus* (*Ch. auratus*) as well as septicaemic wild *O. niloticus* and *C. lazera* (**Hussien *et al.*,1997; El-khatib, 1998 and Jehan, 2001**). This disease pose a continuing threat to pisciculture as even with improved environmental conditions, asymptomatic carriers may become established. Also, dramatically high losses can occur when water conditions are poor or when fish are exposed to stresses. The

prime factors known to evoke subclinical and clinical infections are increased water temperature, low dissolved oxygen, high ammonia, and handling (**Rucker, 1966** and **Bullock and Snieszko, 1979**). Moreover, there is a link between exposure to low levels of copper in the water and clinical disease (**Knittel,1981**). Unfortunately, pond – reared fish may be exposed to different levels of copper as it is sometimes used to treat several bacterial & protozoal diseases and to control algae in fish ponds, besides it probably pollutes the natural water. As a general rule, prevention and control of yersiniosis is impossible without avoiding and correcting the predisposing factors (**Horne and Barnes, 1999**). So, this study was planned to investigate the effect of environmental stressors in promotion of clinical as well as covert yersiniosis among cultured tilapias in Egypt.

MATERIAL AND METHODS

Fish:

A total number of 210 *O. niloticus* weighing 45 ± 5 g. obtained from apparently healthy lots of Fokky fish farm with no history of yersiniosis. They were stocked in 14 glass aquaria (40 x 50 x 100 cm) of 15 fish each. The aquaria were supplied with dechlorinated tap water & continuous aeration. One third of water was changed every two days, two weeks prior to and throughout the period of the study. Fish were not fed during the experiments in order to decrease ammonium production to the water.

Bacterial culture:

A well identified, locally isolated *Y. ruckeri* isolate was kindly supplied by the department of fish diseases, Animal Health Research Institute. Stock cultures were maintained on tryptic soy agar (TSA) slants (Difco, Detroit, Mitchigan)and stored at 4°C. Stock cultures were transferred to fresh slants monthly.

Infection of fish:

100 ml of 24 h old Tryptone soy broth (TSB) culture of *Y. ruckeri* was added to an aerated 20 – liter aquarium. The number of bacteria in the immersion medium corresponded to about 2.5 x 10^{10} colony forming unit (CFU) ml^{-1}. All experimental fish were allowed to remain in contact with the culture for 90 min. Fish in control group were immersed as mentioned above by using bacterial free TSB (**Hietala *et al.*, 1995**).

Water chemistry:

Daily water samples were taken for determination of DO content, pH, NH_3 and hardness. DO and pH were measured by oxygen and pH meters, while NH_3 and hardness were chemically estimated according to **Boyd,**

(1979). During copper exposure, copper sulphate(El- Nasr pharmaceutical Chemical Co.) was used. Water was tested every other day using the Aqua Merck copper test no. 14651, Duplatest CU as noted by **Untergasser, (1989)** for substitution of any missing copper.

Design of stress-inducible Yersiniosis experiment:

The experimental design for exposure of *Yersinia* infected *O. niloticus* to different stressors namely low DO, excess unionized ammonia and various levels of copper were summarized in table.1. All experimental fish were observed over sixty days period for mortalities, behaviour changes, and clinical abnormalities. Dead fish as well as survivors which sacrificed at the end of the experimental period were subjected to post-mortem and bacteriological examinations. Haematological and biochemical investigations were carried out on clinically diseased and control fish.

Bacteriological examination:

Kidney, liver, spleen & intestine swabs were plated onto tryptic soy agar (TSA) and incubated overnight at 30°C for resiolation of *Y. ruckeri*. The isolates were identified by growth on Shotts Waltman (SW) medium (**Shotts, 1991**) and using API 20E system (BioMerieux Vitex, Inc., Hazelwood, MS, USA) as suggested by **Frerichs, (1993)**.

Haematological examinations:

Blood hemoglobin content (Hb), haematocrit (PCV) value and erythrocyte (RBCs) count were assessed according to **Kanaev, (1985)** and **Stoskopf, (1993)**. Blood indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin count concentration (MCHC) were calculated following **Sonnenwirth & Jarett, (1980)**.

Biochemical study:

The Biuret reaction was followed to determine serum total protein levels (**Wotton & Freeman, 1982**).

Statistical analysis:

The obtained data were subjected to the student's t-test (**Gad & Weil, 1986**)

RESULTS

Effect of different stressors on fish mortalities among *Yersinia* infected *O.niloticus*:

Different mortalities were recorded in unstressed and stressed experimentally infected fish (Table 2). Exposure to low DO and sublethal

concentration of copper (1mg/L for 1 month) either at 18°C and 25°C caused 100% deaths in short periods about 17 to 37 days. Similar losses occurred also among infected *O. niloticus* which exposed to copper sulphate in therapeutic doses (1mg/L for 1 hr and 0.15 mg/L for 10 days) at high temperature. Even at 18°C, exposure to copper at pervious doses caused higher mortalities (86.7% and 73.3%) than unstressed ones (60%). Increasing temperature did not affect mortalities greatly either in unstressed and stressed infected fish. The least losses occurred among stressed fish was 13.3 and zero % on exposure to excess NH_3 at 18 and 25°C respectively. Control fish did not show any deaths. A symptomatic mortalities observed mostly among unstressed *O. niloticus* and to a lesser extent in stressed ones. Isolated bacteria from different organs of freshly dead fish were morphologically and biochemically identical to the stock cultures of *Y. ruckeri* as confirmed by plating suspected cultures onto SW medium and production of green colonies with halos which is specific for *Y. ruckeri*. The causative bacteria were recovered from all or most examined organs of dead fish, however, few percentages of some stressed ones revealed *Y. ruckeri* from two organs only (Table. 2). 13.3% of those exposed to copper for 10 days or 1 month coupled with high temperature had *Y. ruckeri* in spleen and intestine. Similarly, *Y. ruckeri* was found in kidney and intestine or kidney and spleen of 26.7 or 20% of those exposed to copper for one month at 18 and 25°C respectively.

Effect of different stressors on forms of Yersiniosis:

Mostly, abnormal swimming & sluggishness accompanied signs of yersiniosis. Skin darkening was the principal clinical signs among yersiniosis affected fish regardless to the presence & type of stressor (Fig.1). Mouth and throat reddening (Fig.2) appeared in relatively few fish especially which exposed to low DO either with or without increased temperature. Also, scale loss, haemorrhages on the external body surface, base of fins and ocular cavity (Fig.3) and iris were seen in stess- inducible yersiniosis. Morbidity percentages increased in some stressed *O. niloticus* to reach 80.7- 100%, except on exposure to copper for 1 hour and 1 month at 18°C where they still similared those recorded among unstressed fish (Table.3). Internally, some stressed fish showed gross congestion of gills, liver, kidneys, spleen, and intestine (Fig.4). Intestine often contained a yellowish fluid. Enlarged spleen and kidneys also were noticed. At the end of the experimental period, asymptomatic survivors' percentages increased on exposure to increased NH_3 at 18 °C and 25°C, decreased on exposure to copper in therapeutic doses at 18°C and disappeared completely in other treatments. All unstressed asymptomatic survivors at 18°C were carriers where *Y. ruckeri* could be reisolated from most organs of them (Table 3). Similarly, covert yersiniosis affected 100% of survived fish which were therapeutically treated with copper at18 °C. In case of exposure to increased NH_3 either at18 °C or25 °C, carriers'

percentages fall to 53.9 and 66.6 °C respectively. On the other hand, no carriers could be detected among unstressed survivors that reared at 25 °C. *Y. ruckeri* was recorded often from kidneys, spleen, intestine and less in the liver No bacteria were recovered from sampled tissues of control fish.

Haematological alterations associated with stress – inducible overt Yersiniosis:

Changes in Hb, PCV, RBCs and blood indices (MCV, MCH and MCHC) of clinically diseased and control fish from all groups were summarized in table.4 .*Y. ruckeri* infection at 18 °C and 25 °C caused significant reduction of Hb. Similarly, all stressors at18 °C produced significant reduction in Hb level, while there were no significant changes at 25 °C. PCV in unstressed fish was nearly similar to control while it generally decreased in stressed fish groups except on stressing by low DO at 18°C and sublethal concentration of copper at 25°C (significantly increased). RBCs count did not significantly affected in all experimental fish except on exposure to excess NH3 at 25°C where it was significantly decreased. Macrocytic and microcytic hypochromic anaemia appeared in unstressed infected fish at 18 °C and 25 °C respectively. Also, macrocytic hypochromic anaemia was detected on exposure to most studied stressors. However, microcytic hypochromic anaemia occurred in ammonia stressed ones either at 18°C or 25°C, low DO at 25°C and copper for 10 days at 18°C.

Serum biochemical findings associated with stress – induciable overt Yersiniosis:

Statistical analysis of total protein indicated its significant reduction in unstressed infected fish at both temperatures (18 °C or 25 °C). Non significant difference between stressed and unstressed *O. niloticus* was observed (Table.4).

DISCUSSION

Although yersiniosis was recognized among wild Nile fishes in Egypt since 1979, no study concerns with such disease among cultured fish up till now. This can be attributed to the similarities between clinical conditions of yersiniosis and other bacterial speticaemic diseases, which may lead to misdiagnosis. Most available literature that dealing with yersiniosis epizootics or its epizootiology concentrated on cold water fish species other than local breeds. So, here, the effect of several stressors which primarily associated with cultured fish in yersiniosis promotion among our farmed *O. niloticus* were fully examined. The results showed that mortality of unstressed infected *O. niloticus* were 53.3-60% within 60 days. **Jehan, (2001)** also recorded 23.3% losses among intraperitoneally infected *O. niloticus* within 21 days. Again **Horne and Barnes, (1999)** reported that over 90% mortality may occur while **Rintamaki *et al.*, (1986)** noted that *Y. ruckeri* infection in

whitefish and Atlantic salmon broodstock caused 0.5 – 4% deaths. Species, susceptibility difference and observation period may be the cause of this variation. On the other hand, some stressors (low DO and copper either at 18°C or 25 °C) increased mortalities than unstressed ones (table.2) . Bullock and **Snieszko, (1979)** and **Knitlel, (1981)** reported nearly similar findings in stressed salmonids. In opposite to the mentioned in the available literature (**Horne & Barnes, 1999**), excess unionized ammonia produced lower deaths (13.3 & zero% at 18 °C & 25 °C respectively). This can be due to difference in NH3 levels and pH, but the increase in the survival of fish than unstressed ones can not be explained. Regarding the effect of temperature, there was no great difference between mortalities at 18°C and 25°C in unstressed and stressed O.niloticus (table.2). Published data about this point disagree with our findings where they unfortunately deal with cold water fish species. Asymptomatic mortality was abundant (7/9 & 6/8 at 18°C and 25°C respectively) in unstressed infected fish. This coincedes with **kawula *et al.*, (1996)** findings in small fry. Oppositely, symptomatic deaths increased with different stressors except on exposure to excess NH3 where the lowest mortality occurred. This can be attributed to the effect of stress factors on the interaction between pathogen and the immune system and other physiological factors of the host (**Horne and Barnes, 1999**). Positive isolation of *Y. ruckeri* from all or even most examined tissues of freshly dead fish means septicaemic state and fish deaths were as a result of the pathogen or combination of stressor and pathogen, not from stressor alone. Behaviour changes, clinical signs and post- mortem lesions which are previously described and shown in (Fig.1&4) were consistent with those reported by **Fuhrman *et al.*, (1983); Rintamaki *et al.*, (1986); Stevenson *et al.*, (1993); Horne and Barnes, (1999)** and **Jehan (2001)**. The reddening of mouth and throat was uncommon among infected O.niloticus. This result confirms the previous observations of **Frerichs *et al.*, (1985)** and **Rintamaki *et al.*, (1986)** in rainbow trout, farmed whitefish and Atlantic Salmon. The increase of morbidity percentages among stressed fish as noticed in this work especially at 25°C has been well documented by **Bullock and Snieszko, (1975)**. He also found that excess ammonia predisposes populations to clinical infection and this disagreed with results presented here. Certainly, only further research can answer, but one may speculate that there are other helping environmental conditions responsible for triggering the clinical infection. The overall forementioned data suggested that yersiniosis in both unstressed and stressed *O. niloticus* appeared in acute and sub-acute forms as previously stated by **Rodriguez *et al.*,** and **Danley *et al.*, (1999)** in rainbow trout and channel catfish. During this study, all infected unstressed and copper exposed survivors at curative doses at 18°C were carriers (Table.3). **Busch and lingg, (1975)** & **Hietala *et al.*, (1995)** also recognized asymptomatic carrier state among rainbow trout by immersion route of exposure. **Frerichs *et al.*, (1985)**

again reported a similar absence of clinical signs in a *Y. ruckeri* epizootic in rainbow trout. **Knittel, (1981)** also found that copper exposure increase susceptibility of fish to *Yersinia* latent infection. *Y. ruckeri* could not be recovered from all unstressed survivors at 25°C after 60 days from initial infection and from 46.1% and 33.4% of ammonia stressed ones at18°C and25°C respectively. **Hunter *et al.*, (1980)** noted that carrier percentage reached undetectable level after 80 days where stress may play an important role in triggering the release of the pathogen from the carrier, therefore stressed fish transmit *Y. ruckeri* to healthy ones. *Y. ruckeri* was readily recovered from one or more of examined organs and kidney, spleen & intestine were the favorable sites for bacterial reisolation from unstressed & stressed survivors. **Busch and lingg, (1975)** added that, during this period liver also can act as a predilection organ. In the meantime, **Jehan, (2001)** recommended the same organs for *Y. ruckeri* isolation from apparently healthy & diseased Nile fishes. The previously mentioned clinical alterations observed in the infected *O. niloticus* could be reflection of significant changes in physicochemical parameters. Significant Hb reduction with macrocytic hypochromic and microcytic hypochromic aneamia in unstressed *Yersinia* infected fish at18°C and25°C respectively agree with **Wobeser, (1973) and Quentel and Aldrin, (1986)** and **Lehman *et al.*, (1987)** observations. Significant and insignificant Hb reduction with macrocytic hypochromic and microcytic hypochromic anaemia were noticed among stressed fish comparing with unstressed ones. This coincedes with previous results which indicated that different stressors increased symptomatic mortalities. Literature concerned with effect of stress exposure on the haematological parameters of yersiniosis – affected fish is nearly lost. However, the recorded haematological changes in stressed infected fish can be attributed to the effect of *Yersinia* endotoxin (**Miller, 1983**) where similar changes were observed in unstressed infected ones. Regarding the total protein it was agreed with **Quentel & Aldrin (1986)** whose value was 2.8 gm/100ml where it decreased than uninfected by 50% approximately which was attributed to cell necrosis found in an infected rainbow trout liver (**Frerichs *et al.*, 1985**). Similar to haematological changes, no reports on total protein changes of stressed infected fish was found. Finally, it could be concluded that Yersiniosis is an incriminated cause of serious economic losses in cultured *O. niloticus* at Egypt. The expectation of yersiniosis troubles mostly, is in or near summer where temperature rises (18-25°C). Fish would succumb to infection if sufficient husbandry stress were applied. So, good husbandry is essential for controlling yersiniosis to acceptable levels .Also, copper sulphate use in pond-reared fish should be avoided to great extent especially where yersiniosis is endemic.

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Table (1): Design and water parameters of stress inducible yersiniosis experiment.

Fish group	Aquar. Number	Infection by Y.rucheri	Water temp. °C	Mean Do (mg/L)	Mean NH_3 (mg/L)	PH	Mean copper concentration (mg/L)	Mean water hardness as $CaCO_3$ (mg/L)	Time of copper exposure
I	1	No inf.	18	7.5	0.013	8.0	*	177.9	-
	2		25	6.85	0.018	8.2	*	177.9	-
II	3	inf.	18	7.5	0.013	8.0	*	177.9	-
	4		25	6.85	0.018	8.2	*	177.9	-
III	5	inf.	18	5.5	0.024	8.3	*	177.9	-
	6		25	4.7	0.033	8.5	*	177.9	-
IV	7	inf.	18	7.5	0.040	8.6	*	177.9	-
	8		25	6.85	0.052	8.8	*	177.9	-
V	9	inf.	18	7.5	0.013	8.0	1.0	177.9	1 hr
	10		25	6.85	0.018	8.2	1.0	177.9	1 hr
VI	11	inf.	18	7.5	0.013	8.0	0.15	177.9	10 days
	12		25	6.85	0.018	8.2	0.15	177.9	10 days
VII	13	inf.	18	7.5	0.013	8.0	1.0	177.9	1 month
	14		25	6.85	0.018	8.2	1.0	177.9	1 month

Aquar. = Aquarium Inf. = Infection Temp = Temperature *= Undetectable

Table 2 : Percentages of mortalites and infected organs in uninfected, unstressed & stressed Yersinia infected *O.niloticus*

Fish group	Aquar. Number	Infection by Y.ruckeri	Stressors	Water Temp. °C	Rate of mortalities			Mortality %	EXP. Period (days)	% of dead fish having infected organs			
					Asymptomatic	Symptomatic	Total			L,S,K & I	K+I	S+I	K+S
I	1	NO Inf.	No	18	0/15	0/15	0/15	0.0	60	0	0	0	0
	2			25	0/15	0/15	0/15	0.0	60	0	0	0	0
II	3	Inf.	No	18	7/15	2/15	9/15	60.0	60	100	0	0	0
	4			25	6/15	2/15	8/15	53.3	60	100	0	0	0
III	5	"	Low DO	18	2/15	13/15	15/15	100.0	17	100	0	0	0
	6			25	3/15	12/15	15/15	100.0	33	100	0	0	0
IV	7	"	High NH_3	18	2/15	0/15	2/15	13.3	60	100	0	0	0
	8			25	0/15	0/15	0/15	0.0	60	0	0	0	0
V	9	"	Copper 1 mg/L for 1 hr	18	7/15	6/15	13/15	86.7	60	100	0	0	0
	10			25	0/15	15/15	15/15	100.0	19	100	0	0	0
VI	11	"	Copper 0.15 mg/L for 10 days	18	0/15	11/15	11/15	73.3	60	100	0	0	0
	12			25	0/15	15/15	15/15	100.0	37	86.7	0	13.3	0
VII	13	"	Copper 1 mg/L for 1 M	18	7/15	8/15	15/15	100.0	30	73.3	26.7	0	0
	14			25	0/15	15/15	15/15	100.0	30	66.7	0	13.3	20

Inf.= Infection hr = hour L = Liver K = Kidney
temp = Temprature M = Month S = Spleen I= Intestine

Table 3: Percentages of morbidity, carrier and recovery in uninfected, unstressed & stressed *Yersinia* infected *O.niloticus*

Fish group	Aquar. Number	Infection by Y.ruckeri	Stressors	Water Temp. °c	N. of moribund tilapias showed			Morbidity rate	Morbidity %	rate of Asymptomatic survivors	Asymptomatic survivors %	% of asymptomatic survivors having infected organs							Carrier %	Recovery%
					Skin darkening	Mouth & throat redding	Other signs of Septicaenia					L	K	I	K+I	S+I	K+S+I	L+K+S+I		
I	1	NO Inf.	No	18	0	0	0	0/15	0	0/15	0	0	0	0	0	0	0	0	0	0
	2			25	0	0	0	0/15	0	0/15	0	0	0	0	0	0	0	0	0	0
II	3	Inf.	No	18	8	0	0	8/15	53.3	6/15	40	16.7	16.7	0	0	33.3	33.3	0	100	0
	4			25	6	3	0	9/15	60	7/15	46.7	0	0	0	0	0	0	0	0	100
III	5	"	Low DO	18	12	1	0	13/15	86.7	0/15	0	0	0	0	0	0	0	0	0	0
	6			25	9	3	0	12/15	80	0/15	0	0	0	0	0	0	0	0	0	0
IV	7	"	High NH3	18	0	0	0	0/15	0	13/15	86.7	0	0	38.5	0	15.4	0	0	53.9	46.1
	8			25	0	0	0	0/15	0	15/15	100	0	13.3	33.3	0	0	0	20	66.63	33.4
V	9	"	Copper 1 mg/l for 1 hr	18	6	0	2	8/15	53.3	2/15	13.3	0	0	0	0	0	0	100	100	0
	10			25	15	0	0	15/15	100	0/15	0	0	0	0	0	0	0	0	0	0
VI	11	"	Copper 0.15 mg/l for 10 days	18	15	0	0	15/15	100	4/15	26.7	0	0	0	25	25	50	0	100	0
	12			25	15	0	0	15/15	100	0/15	0	0	0	0	0	0	0	0	0	0
VII	13	"	Copper 1 mg/l for 1 M	18	3	2	3	8/15	53.3	0/15	0	0	0	0	0	0	0	0	0	0
	14			25	15	0	0	15/15	100	0/15	0	0	0	0	0	0	0	0	0	0

Inf.= Infection hr = hour L = Liver K = Kidney
temp = Temprature M = Month S = Spleen I= Intestine

Table 4 : Means of Hb, PCV, RBCs, blood indices and total protien ± SD in uninfected, unstressed &stressed Yersiniosis diseased ***O.niloticus***

Fish group	Aquar No	Infection by Y. ruckeri	Stressors	Temp °C	Hb conc (g/dl)	PCV Value%	RBCs Count 10 /mm	MCV% (FL)	MCH% (Pg)	MCHC%	T.P (g/dl)
I	1	No	No	18	8.95± 0.29	15.5± 0.58	1.86± 0.18	84.19± 11.47	48.6± 6.38	57.6± 0.46	4.9± 0.11
	2			25	6.8± 0.1	17± 2.3	1.5± 0.4	194.2± 38.2	49.7 ±13.1	65.3 ±1.7	4.5± 0.4
II	3	Inf	No	18	8 ±1.6 T 3.3 **	15.8± 5.2 T 0.4	1.6± 0.6 T 0	104.3± 25 T 3.9***	32.9± 6.4 T 6.8****	52.3± 6.4 T 6.3****	2.4± 0.4 T 2.5*
	4			25	4.9± 2.3 T 3.8 ***	17.3 ±7.7 T 0.3	2.12± 0.17 T 0.6	81.1± 25.2 T 8.3****	25± 4.1 T 5.5****	52.5 ±6.6 T 19.4****	2.6± 0.9 T 2.2*
III	5	Inf	LowDO	18	6.8± 0.7 T 3.0 **	19.5 ±4 T 2.5 *	1.3± 0.1 T 0.3	153± 20.1 T 6.4****	53.7 ±1.3 T 20.8****	35.5± 3.8 T 4.5****	1.8± 0.3 T 0.6
	6			25	5.6 ±1.4 T 1.0	13.7± 7.5 T 1.0	2± 0.4 T 0.1	65± 29.2 T 1.0	27.7± 2.4 T 2.0	50.4 ±25.6 T 1.0	2.1± 0.8 T 1.7
IV	7	Inf	High NH3	18	5.3± 0.8 T 6.8 ****	13.4± 3.6 T 2.2 *	1.5± 0.1 T 0	88.7± 21.8 T 2.4*	35.1± 6.1 T 0.7	40.7± 6.3 T 4.7****	2.9± 1.8 T 1.3
	8			25	4.8 ±1.3 T 0.2	12± 3.7 T 2.7 *	1.5± 0.5 T 6.0 ****	73 ±13.2 T 1.2	32.4± 7.1 T 3.0***	41.3± 8.4 T 6.8****	2.4 ±0.2 T 0.2
V	9	Inf	Copper 1 mg/L for 1 hr	18	4.2± .01 T 7.6 ****	14.5± 2.9 T 1.1	1.6± 0.8 T 0	126±85.5 T 1.0	36.8± 25.5 T 0.5	28.9± 0.6 T 14.8****	2.6± 0.8 T 1.0
	10			25	5.1± 0.1 T 0.5	14.5± 2.9 T 1.6	1.15 ±0.4 T 1.0	141.4± 67.7 T 2.6*	46.7 ±14 T 4.5***	35.6± 7.1 T 10.0****	1.8± 0.2 T 1.0
VI	11	Inf	Copper 0.15 mg/L for 10 days	18	5.3± 1.9 T 6.8 ****	14.6± 5.6 T 1.0	1.8± 0.8 T 0.2	91.7± 48 T 1.4	32.7 ±14.4 T 0.1	38.1± 9 T 3.4***	2.1± 0.4 T 0.3
	12			25	5.9± 0.7 T 2	16.3±2.2 T 0.6	1.7± 0.3 T 0.4	94.7± 3.4 T 2.6*	34.4 ±1.8 T 9.4****	36.4± 0.6 T 20****	2.6± 0.5 T 0
VII	13	Inf	Copper 1 mg/L for 1 M	18	4.1± 1 T 7.8 ***	11± 2.3 T 4.8 ****	0.63± 0.01 T 0.6	174.2 ±33.4 T 6.0****	64.9± 15.3 T 6.2****	37.1± 0.7 T 5.2****	1.3± 0.4 T 1.1
	14			25	6.3±1.5 T 1.6	21± 7 T 1.0	2.4± 1 T 2.0	88.7± 9.5 T 1.1	28.2± 8.4 T 0.7	31.3± 6.2 T 9.2****	3.4± 0.2 T 1.0

T= test

* Significant Difference P value < 0.05

**Significant Difference P value < 0.01

*** Significant Difference P value < 0.005

****Significant Difference P value < 0.001

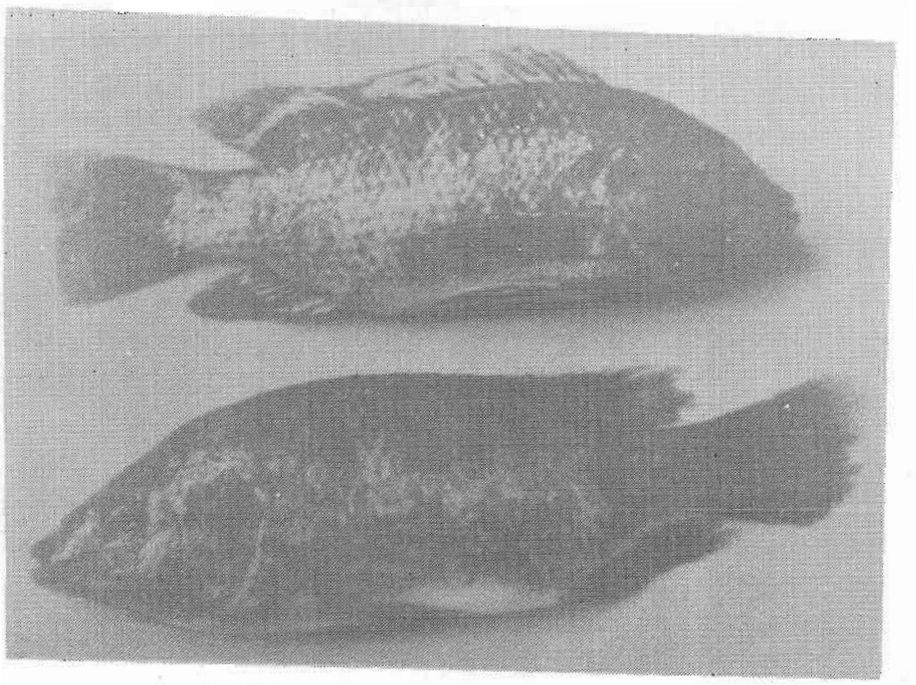

Fig. (1): (Above) moribund *O. niloticus* species exposed to copper for 10 days at 18 °C post *Y. ruckeri* infection showing skin darkening.(Below) moribund *O. niloticus* species exposed to copper(1 mg/L) for 1 month at 25 °C post *Yensinia* infection showing skin darkening and tail and fin rot.

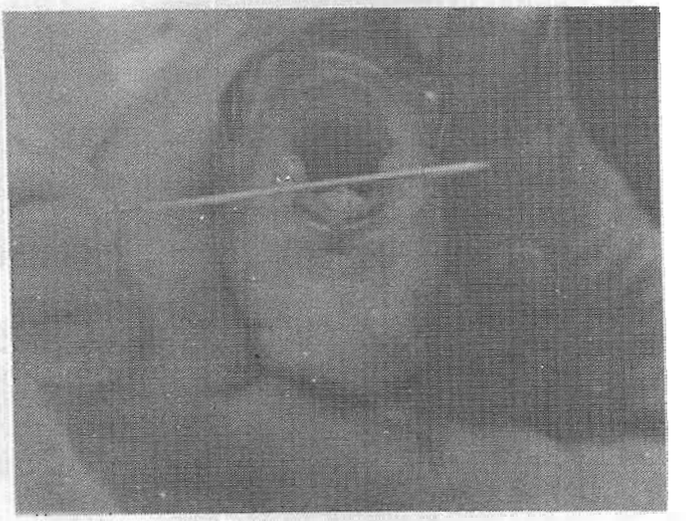

Fig. (2): Moribund *O. niloticus* species exposed to low DO(0.033 mg/L) at 25 °C post *Y. ruckeri* infection showing mouth and throat redding.

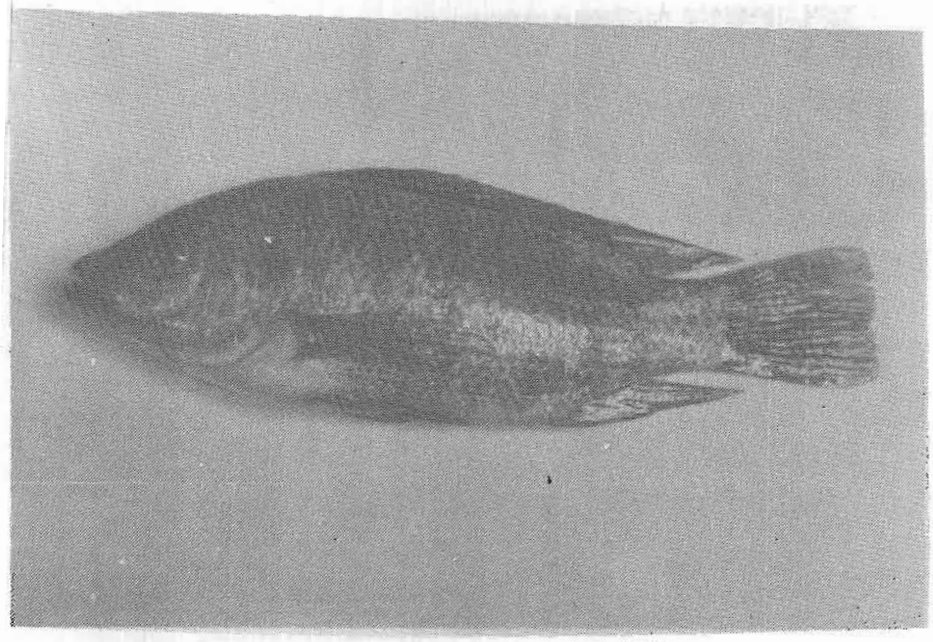

Fig. (3): Moribund *O. niloticus* species exposed to copper (1 mg/L for 1 month) at 18 ° C post *Y. ruckeri* infection showing congestion of head region and haemorrhages at fin bases.

Fig. (4): Moribund *O. niloticus* species exposed to low DO (0.033mg/L) at 25°C post- *Y. ruckeri* infection showing congestion of gills, liver & intestine.

الملخص العربي

إستكشاف دور الإجهاد في تشجيع الإصابة باليارسينوزس في أسماك البلطي النيلي المستزرعة في مصر

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أجريت هذه الدراسة لمعرفة تأثير التعرض للعديد من العوامل المجهدة على تشجيع إصابة أسماك البلطي المستزرعة باليارسينوزس . لذلك تم تعريض أسماك البلطي المعداة باليارسنييا روكرى لنقص في الأكسجين الذائب , زيادة في الأمونيا الغير متأينة ، مستويات مختلفة من النحاس (١ مجم / لتر لمدة ساعة ، ٠,١٥ مجم / لتر لمدة ١٠ أيام ، ١ مجم / لتر / شهر) عند درجتي حرارة مياه ١٨°م، ٢٥°م . وقد لوحظ حدوث زيادة في نسبتي النفوق و الإصابة في الأسماك المعرضة لنقص في كمية الأكسجين الذائب أو للنحاس عند درجات الحرارة السابقة عن الأسماك الغير معرضة لتلك العوامل . وقد ظهر مرض اليارسينوزس في الصورة الحادة أو تحت الحادة وكانت الأسماك الحية في نهاية التجربة سليمة ظاهريا ولكن أغلبها كان حاملا للمرض . وقد كانت العلامات الإكلينيكية الرئيسية في الأسماك المصابة هي دكانة الجلد مع أو بدون بعض علامات التسمم الدموي الأخرى. أقل مجموعة حدث فيها نفوق مع بقاء الأسماك دون ظهور أية تغيرات إكلينيكية هي المجموعة التي تعرضت لزيادة في نسبة الأمونيا عند درجتي حرارة مياه (١٨°م، ٢٥°م) وقد حدث انخفاض معنوي في الهيموجلوبين والبروتين الكلى في مصل دم الأسماك المصابة الغير مجهدة كما أن كل العوامل المجهدة سببت أنيميا حادة مع انخفاض غير معنوي في البروتين الكلى .